# UTILISATION OF ANIMAL WASTES AND SEWAGE SLUDGES AND THEIR SUBSEQUENT EFFECTS ON HIGHER TROPHIC LEVELS IN FOOD CHAINS

a thesis submitted as partial fulfilment for the degree of Master of Philosophy

by

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I would also like to devote this piece of work to my wife, Margaret, who gave me spiritual support and continuous encouragement.

ABSTRACT

1 When comparing the properties of the waste materials, it was revealed that activated sludge had the lowest content of total nitrogen (0.59%) and extractable phosphate (28 ppm) followed by digested sludge, chicken manure and pig manure.

Digested sludge had the highest level of total Pb (33 ppm), Cu (105 ppm) and Zn (753 ppm) while chicken manure had the highest level of total Mn. However, when considering the exchangeable contents of heavy metals, all the waste materials were similar except that both the sewage sludges had significantly higher ($p < 0.05$) level of Zn (activated sludge : 139 ppm, digested sludge : 87 ppm) than the animal manures (chicken manure : 18 ppm, pig manure : 14 ppm). The animal manures also contained a significantly higher ($p < 0.05$) content of organic carbon (chicken manure : 33%, pig manure : 48%) than sewage sludges (activated sludge : 6%, digested sludge : 10%).

2 Aqueous extracts of activated sludge, digested sludge, chicken manure and pig manure were used to cultivate <u>Chlorella pyrenoidosa</u> in the laboratory. Cell number, oven-dried weight, chlorophyll and heavy metal contents were measured. Activated sludge extracts produced an algal product with the lowest oven-dried weight (1% extract : 6 mg/100 ml), and highest content of Cu (1% extract : 3,656 ppm) and Zn (1% extract : 6,604 ppm). Animal manures were

more suitable for algal growth as indicated by their higher oven-dried weight (1% chicken manure extract : 61 mg/100 ml, 1% pig manure extract : 43 mg/100 ml) and lower level of Cu (1% chicken manure extract : 380 ppm, 1% pig manure extract : 363 ppm) and Zn (1% chicken manure extract : 493 ppm, 1% pig manure extract : 319 ppm) in the algal products.

3 None of the wastes was found satisfactory when used as supplementary feeds for the common carp, Cyprinus carpio. All the fish fed with waste materials lost weight and both the animal manures caused death of the fish during a culture of 8 days. Furthermore, fish fed with activated sludge accumulated higher levels of Pb, Cu and Cd. Those fed with animal manures accumulated higher level of Zn and Mn in the flesh.

4 The waste materials were applied to soil for growing Flowering Chinese Cabbage, Brassica parachinenses. It was discovered that animal manures supported superior growth which was reflected by the higher productivity, earlier maturity and lower heavy metal contents of the crops.

Among the three portions of the vegetable analysed, the level of heavy metal content in a descending order was root, shoot and leaf. The inferior growth of the vegetable growth with sewage sludges was found correlated with the edaphic properties of the sewage sludge-amended soils. Such soils were found lower in soil pH, conductivity and extractable phospate but higher in heavy metal contents.

5 The simulated aquatic food chain involving the four waste materials, unicellular green alga (Chlorella pyrenoidosa) and freshwater shrimps (Palaemonetes sp.) demonstrated that shrimps fed with sewage sludge-grown algae had a lower increase in body weight (activated sludge : 6 - 8%, digested sludge : 17 - 23%) and a higher level of heavy metals (activated sludge : Pb 217 ppm, Cu 2,403 ppm, Zn 3,055 ppm and Mn 360 ppm; digested sludge : Pb 61 ppm, Cu 851 ppm, Zn 942 ppm and Mn 145 ppm). Higher increase in body weight (chicken manure : 36 - 39%, pig manure : 42 - 45%) and a lower level of heavy metals (chicken manure : Pb 49 ppm, Cu 496 ppm, Zn 742 ppm and Mn 334 ppm; pig manure : Pb 73 ppm, Cu 360 ppm, Zn 497 ppm and Mn 172 ppm) were found in those fed with animal manure-grown algae.

The heavy metal contents (Pb, Cu and Zn) were found significantly correlated (p 0.01) with that in the algae used as food.

Moulted exoskeleton of the shrimps had a higher level of all the metals suggested that molting might serve as a bioelimination mechanism for discharging toxic heavy metals.

6 The simualted terrestrial food chain involving the four waste materials, vegetables (Flowering Chinese Cabbage, Brassica parachinensis and Chinese Radish, Rhaphanus sativus var. longipinnatus) and caterpillars of Common White Butterfly (Pieris canidia) also showed that caterpillars fed with

sewage sludge-grown vegetables had a lower body weight and a higher level of heavy metals than those fed with animal manure-grown vegetables.

Caterpillars even though ingested an enormous amount of the leaves accumulated a lower heavy metal level that that found in the vegetables. Nevertheless, the route of elimination was not verified but it was suggested the egestion of faeces might play an important role.

CONTENTS

# CHAPTER ONE

## Introduction

### 1.1 PRESENT SITUATION OF WATER POLLUTION PROBLEM IN HONG KONG

Being a predominantly industrial community in a very confined space and having high population density, Hong Kong is located at the Po On District of the Kwantang Province of China. The total area of 1,060 $km^2$ (Hong Kong Government, 1981) includes the Hong Kong Island, Kowloon Peninsula, New Territories and more than two hundred out-lying islands.

The total estimated population size at the end of 1980 was 5,147,900. This population makes Hong Kong one of the most densely populated places in the world with an overall density of 4,852 $km^{-2}$. However, the population density varies in different areas.

According to the 1976 by-census of Hong Kong, Kowloon and Tsuen Wan was 25,400 people $km^{-2}$. But for the New Territories, it was 554 $km^{-2}$.

The average annual rate of increase in population over the past 10-year period was 2.5%. This everincreasing population size has introduced changes to the environment owing to the increase in domestic, agricultural and industrial activities. Following such activities, it comes the problems of waste disposal. Such wastes have been contaminating our surrounding water environment and these wastes can be catagorized into three groups :

A Domestic wastes

The direct passage of large majority of domestic sewage effluents into the Victoria harbour only after preliminary screening has been a general practice. Such outfalls bring along with human faecal bacteria and infectious pathogenic bacteria to the seashores around the coastline. Seafood (including clams and oysters) collected in coastal seashores is possible to feed in and transfer these pathogens to people eating them.

In 1974, local people were found suffering from infectious hepatitis after eating such contaminated molluscs. The oysters carrying the disease were collected from Deep Bay where the water was contaminated by domestic and industrial sewage, probably from Yuen Long.

Another case study had been conducted on the level of concentration of coliform bacteria in clams from Tolo Harbour. The safety level should be 15 coliforms bacteria per gram of the animal. However, 825 - 11,000 coliforms bacteria were found per gram of dried tissue. This really indicates the severe degree of sewage pollution which affects the seashore animals (Wong et al., 1977b).

B Agricultural wastes

People in Hong Kong are mainly Chinese who have a strong preference of freshly slaughtered meat and fish, and also for fresh vegetables. The estimated local production of livestock in 1979 included half a million pigs and six millions chickens

(Agriculture and Fisheries Department, Hong Kong, 1980a).

It is estimated that each pig produces double amount readily putresible matter as one human being and twelve chickens produce about the same amount as human being. Thus the above estimated amount of livestock produce wastes equivalent to 1.45 million of human being population. The human population of the New Territories is some 400,000. Thus, 'population equivalent' of the population from livestock is therefore substantially greater than that of the inhabitants of the area (Isaac and Revell, 1977).

In 1974, Bine and Partners conducted a survey on the types and importance of pollutants entering streams in the New Territories. It was found that agricultural effluents from pig farms contribute 54.2% of the pollutants while poultry droppings contributes 13.4% of the pollutants (Table 1.1).

It was estimated that the total BOD (biochemical oxygen demand) reaching Hong Kong freshwater streams was some 52,000 kg/day (Environmental Resources Limited, 1977). Out of which, 86% is contributed by agriculture. Probably, four-fifth of this pollution comes from pigs and the rest from poultry (Wong et al., 1979a).

Pig farmers build their pig styes near waterstreams where the disposal of the manure in slurry form is made easy by simply flushing the houses with water. Therefore, wastewater leaving the houses carries the excreta into the waterstreams without any form of treatment.

Table 1.1 Types and importance of pollutants entering streams in the New Territories, Hong Kong (from Bine and Partners, 1974)

| | % |
|---|---|
| Liquid waste | |
| Domestic sewage, sullage and night-soil | 10.2 |
| Industrial effluents | 21.7 |
| Agricultural effluents from pig farms | 54.2 |
| Solid wastes | |
| Domestic refuse | 0.4 |
| Industrial solid wastes | 0.1 |
| Agricultural solid waste - poultry droppings | 13.4 |
| | 100.0 |

Poultry manure, usually in semi-solid form, is more readily recycling than pig manure. However, only about 30% of such production is being recycled. It is used as a fish pond fertilizer in Hong Kong. Besides, chironomid larvae are harvested from shallow water fields enriched with chicken manure for aquarium fish and carnivorous fish fry (Shaw and Mark, 1981) (Figs. 1.1 and 1.2). The rest is improperly dumped and finally contaminates the watercourses (Agriculture and Fisheries Department, Hong Kong, 1980b). The reason is that collection and re-utilization of this waste is economically unfavourable when compared with the use of artificial fertilizers.

The usage of chemical fertilizers by farmers has further complicated the water pollution problem. These fertilizers have high levels of nitrogen (N), phosphorus (P) and potassium (K) which, when in excess of crop uptake, are washed into waterstreams. Enrichment of these macroelements in the receiving water will cause eutrophication. 'Red-tide' - an out-break of phytoplankton in eutrophicated water usually takes place in Tolo Harbour at the entrance to Tai Po Hoi during the spring rains (Short, 1980). Such out-break depletes oxygen level in the harbour and finally affects the fish population.

Beside chemical fertilizers, pesticides are also widely used nowadays in agricultural activities. Pesticides can be classified into groups of organochlorides, organophosphates,

Fig. 1.1 Village women are harvesting their chironomid larvae from shallow fields enriched with poultry manure

Fig. 1.2 The washed product contains the larvae and fine mud which need further cleaning before selling as fish food

carbamates and naturally occuring ones. Out of them, DDD, DDT, aldrin, etc. belong to the group of synthetic organochlorides of chlorinated hydrocarbons. This group of pesticides are non-biodegradable, persistent in environment and fat soluble. Incorrect or excessive use of such chemical compounds in fields will contaminate our ecosystem. Edible corps, farm animals and even fishes may be contaminated through biological concentration.

Animal manures, chemical fertilizers and pesticides are therefore causing considerable water pollution in streams and rivers in the low-lying areas in the north-western part of New Territories.

C Industrial wastes

A Water Pollution Ordinance was enacted on July 1980. Before this, there is no restriction to prohibit the discharge of untreated industrial effluents to the water environment except in catchment areas.

Composition of these industrial effluents varies according to the different kinds of industries. However, these effluents generally process two properties which affect the quality of the receiving water. Those discharged from food, beverage and textile industries have biochemical oxygen demand considerably high and deplete the the oxygen level in the receiving water quickly, making it unsuitable for living organisms. Another group of discharges comes from dyeing, electroplating, photoprocessing, tannery and metal finishing industries. Such effluents have high levels of toxic complex chemical substances including various heavy metals.

Living organisms in the receiving water usually cannot tolerate such toxic substances. A good example is demonstrated by the previously presence of tanneries in Sheng Shui. It was noted that no living organism could be found in the Sutlej River which receives the tannery effluents (Wong, 1979b). The reason is that such effluents contain chromium (Cr) which is highly toxic.

In some cases, organisms in the receiving water may tolerate the toxic substance and keep on living in the polluted water. However, edible organisms in such cases may accumulate such toxic substances in their body during their period of growth. Cadmium (Cd) contamination of the Pacific oyster (Crassostrea gigas) cultured in Deep Bay provided a good evidence in this aspect (Wong et al., in press).

## 1.2 HISTORY OF ENVIRONMENTAL POLLUTION CONTROL IN HONG KONG

According to its geographic location, Hong Kong has avoided, to a great extent, severe water pollution problems in its water because of the effectiveness of coastal currents (Hong Kong Government, 1980). Such coastal currents include the North China Coastal Water and South China Sea Water in winter season and the Pearl River in summer season (Williamson, 1970) (Fig. 1.3).

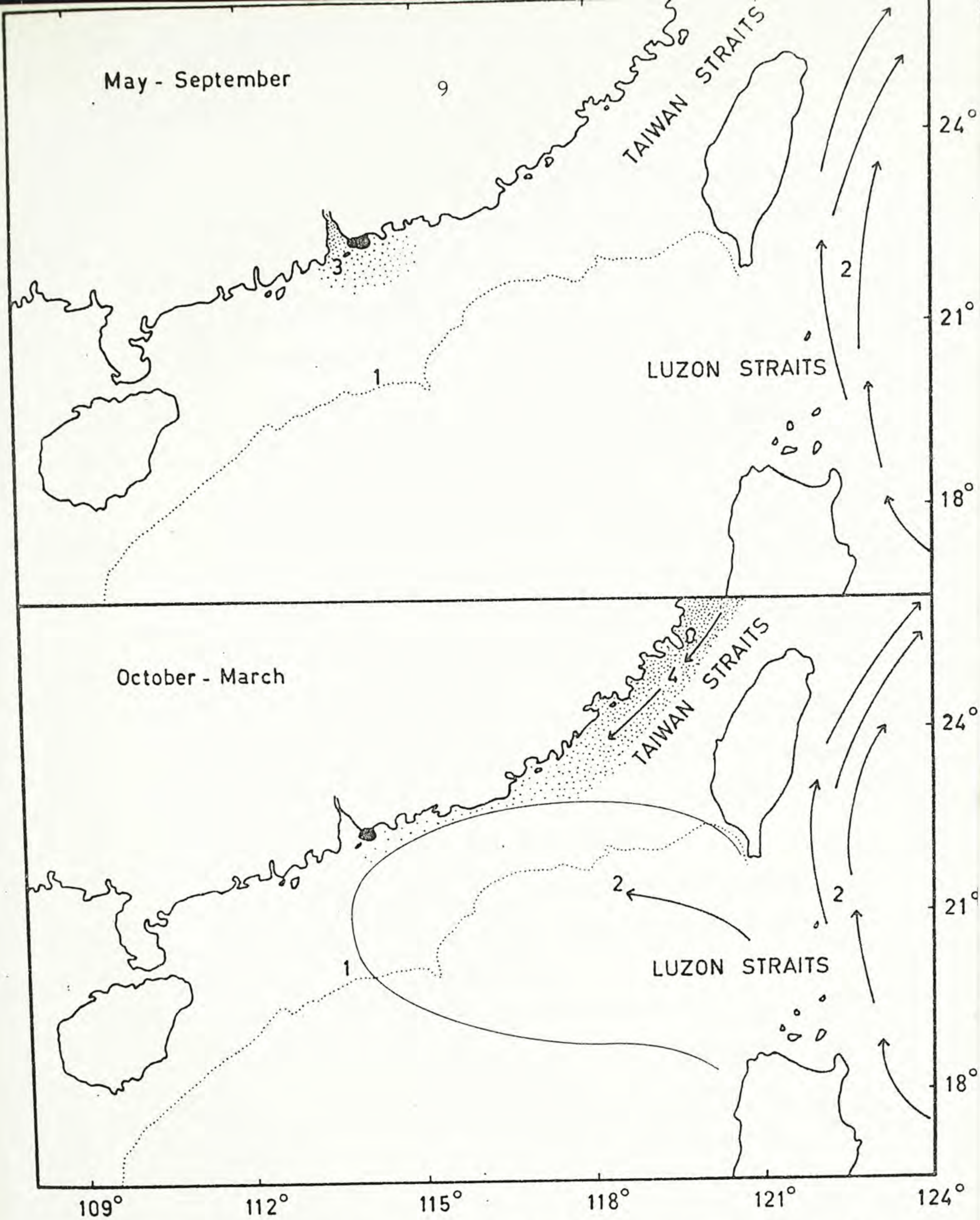


Fig. 1.3 Influence of different water masses in the northern part of the South China Sea. 1. South China Sea Water. 2. Kuroshio Water. 3. Fresh Water. 4. North China Coast Water. (From Williamson, 1970)

However, a closer look at the map of Hing Kong will show that ther are still some 'dead spaces' wher coastal water currents cannot efficiently function. These regions (Fig. 1.4) includes :

1 North West Kowloon region from Yau Ma Tei to Mei Foo Sun Chen,

2 Region between Kwun Tong and the reclamated eastern end of Kai Tak Airport,

3 Western end of Tolo Harbour near Tai Po (Short, 1980).

Environmental Resources Limited, was appointed by the Government in 1974 to propose legislations required to protect the environment of Hong Kong. Then in 1977, a final report 'Control of the Environment in Hong Kong' was presented and five new ordinances were proposed. These ordinances include Water Pollution, Air Pollution, Noise Pollution, Solid Wastes Pollution and Environmentsl Impact Statements.

After this, drafting of new environmental protection legislations began in 1978. The Environmental Protection Agency (an organization attached to the Environmental Branch under the Secretary for the Environment) handled the Environmental Pollution Control policy development and environmental monitoring. In 1980, Government has enacted legislation for the Water Pollution Control Ordinance. The purpose of this ordinance is to limit liquid effluent discharges which are not controlled before. Under such ordinance, all liquid effluent dischargers will be licensed

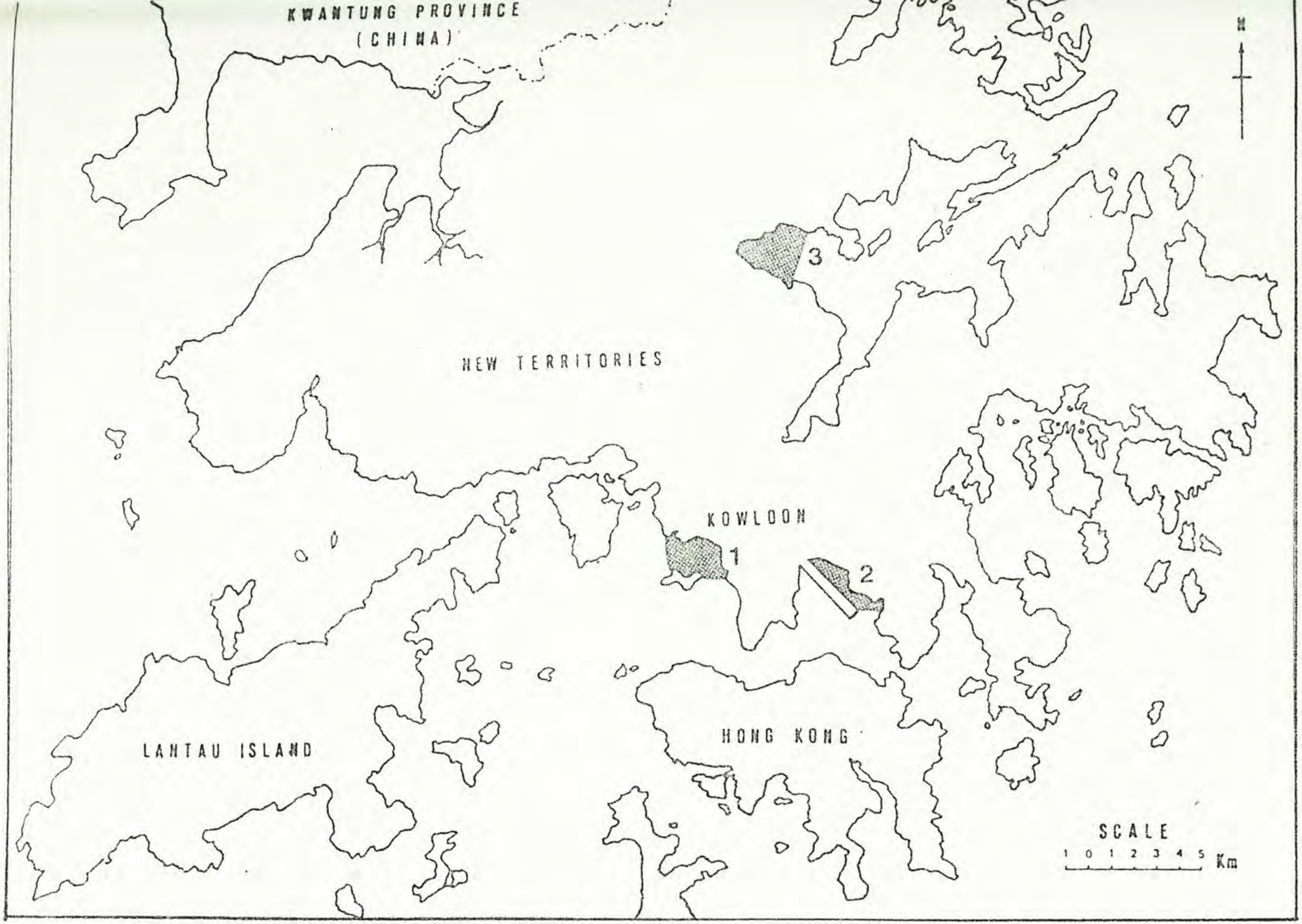


Fig. 1.4 Three 'dead spaces' where coastal currents cannot efficiently function (from Short, 1980).

and different control standards will apply in different areas (Environmental Branch, Hong Kong, 1979).

## 1.3 CONTEMPORARY STATUS IN WASTEWATER AND SOLID WASTES MANAGEMENT IN HONG KONG

### A Domestic wastes

Public Works Department (PWD) is responsible for the development of sewage works and sewage treatment plants to handle the large amount of sewage outfall from urban areas. In the year of 1974, a Pilot Sewage Treatment Plant was established at Shek Wu Hui of Fanling to compare the efficiencies of four separate biological treatment processes of sewage which include pure oxygen activation method, air activation method, biological filtration method and oxidation pond method. All these methods are primarily aimed at the removal of suspended solids in the wastewater. Finally, the solids are concentrated and removed as sewage sludge. In Shek Wu Hui Sewage Treatment Plant, sewage sludge is further digested in an anaerobic digestion tank and the final product is called digested sludge. In The Sewage Treatment Plant of The Chinese University of Hong Kong, sewage sludge is dried on sand bed. The final product is called activated sludge (since activation method is the only method used in this sewage treatment plant for treating the wastewater).

Sewage sludge has properties depending on the wastewater sources and the methods of treatment applied. With the advancement in technology of sewage treatment, the treated effluent has a better water quality but the sewage sludge produced has a higher level of the pollutants removed. This introduces the problem of disposal of the sludges.

There are only three main sewage treatment plants in Hong Kong. These include the Shek Wu Hui Pilot Treatment Plant, The Chinese University Sewage Treatment Plant and the Shatin Temporary Sewage Treatment Plant. Further expansion of sewage treatment works will include the establishment of advance sewage treatment works in new towns of Shatin, Tai Po and Tuen Mun (Hong Kong Government, 1981).

B Agricultural wastes

Agriculture and Fisheries Department (AFD) of Hong Kong estimated that more than 1,300 tonnes of pig manure and poultry manure are being discharged into the rivers daily (Agriculture and Fisheries Department, Hong Kong, 1980). On the other hand, the annual consumption of wash water for pig and poultry farms is about 10 million $m^3$, enough to fill the Jubilee Reservoir.

Some of the streams and rivers which were formerly collecting water in the catchment areas into our reservoirs has now been polluted by the manure, rendering it no longer suitable for the catchment of water. Evidence can be cited in the Tai Po Tau Fabric Dam Station (Figs. 1.5, 1.6). The station, by means of the fabric dam and the central

Fig. 1.5 Fabric dam station at Tai Po Tau, the big round structure behind the fibric dam is used to collect water in this catchment area to the Plover Clove Reservior

Fig. 1.6 Now the water is excessively enriched with pollutants and nutrients from farm wastes

collecting hole, collects water from the catchment area into the Plover Clover Reservoir. Now the watercourses in the catchment area has been eutrophicated with algal bloom.

Contemporary, AFD adopts the following two methods in handling the pig and poultry manure :

a Manure collection service

AFD has two stations in New Territories to dry the collected animal manures. One is at Pat Heung for the drying of chicken manure. The other one is in Sai Kung for the drying of pig manure. Chicken manure is collected by a private contractor for the Pat Heung Plant at a cost of $45 per ton (Fig. 1.7). The final dried product is sold in plastic bags at the subsidized prize of $15 per 10 kg (Agriculture and Fisheries Department, Hong Kong, 1980b) (Fig. 1.8). Dried poultry manure is a good fertilizer for gardening purposes. Nevertheless, the collection service can only handle 5 and 30% of pig manure and poultry manure respectively. The major portion is still handled inadequately by the farmers themselves.

b Supervisory service

AFD is now trying to educate the farmers about the subsequent effects of their current methods of handling their animal manures and the following methods are suggested :

Fig. 1.7 Poultry manure collected is first stored in bunkers for a few days in order to lower the moisture content

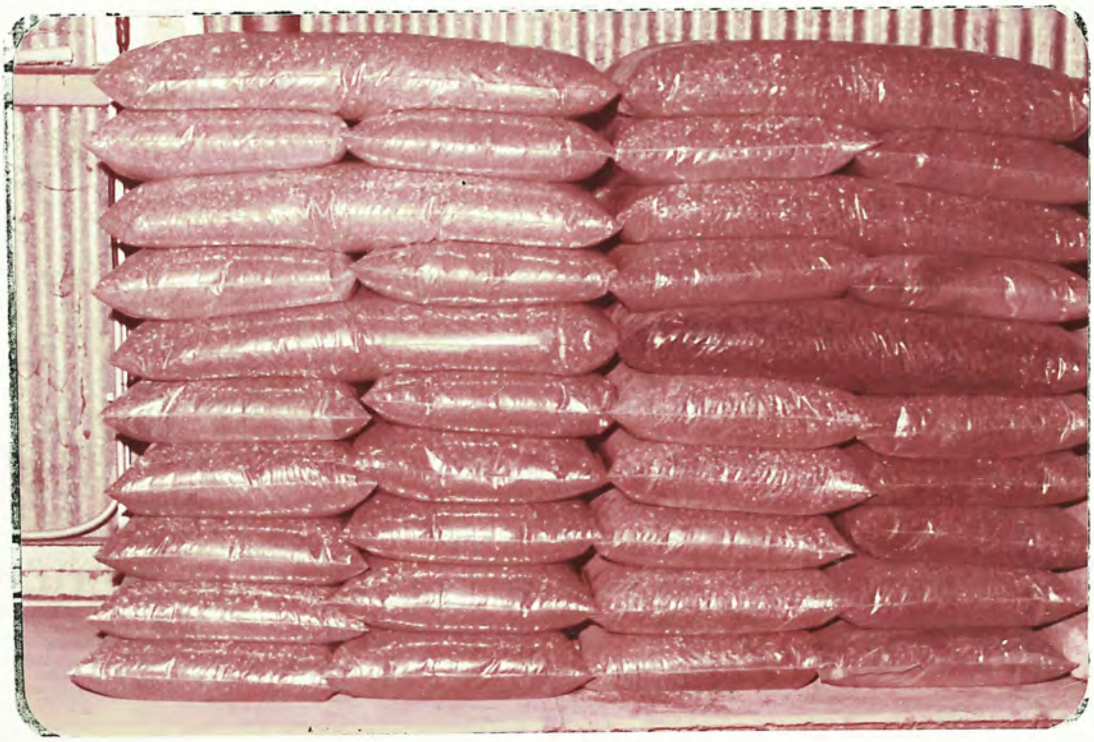

Fig. 1.8 The treated poultry manure is favoured by local farmers

i Solid Treatment method

Farmers are adviced not to flush their pig farms with water but 'muck out dry' (Fig. 1.9). Pig faeces should be swept together when it is still dry. Swept faeces can be collected by basket in pits of drains, liquid portion of the faeces will drain off from the basket to the rocks paved pit. Semi-dried faeces in the basket can be stored in bunkers for a few days allowing to dry out part of its water content. The partially dried faeces can later be transported to collection centres for complete drying.

ii Effluent Treatment methods

Two methods are now being used to treat the effluent from the pig styes in the Ta Ku Ling Station of AFD.

Aeration method (Fig. 1.10) incorporates an aeration tank and a sedimentation tank. The tanks should be large enough to handle 4 days' effluent from the farm. An eletric driven motor is used to aerate the collected effluent throughly thus allows aerobic digestion of the effluent. Sedimentation in the co-ordinating tank is required for the separation of solid after aeration.

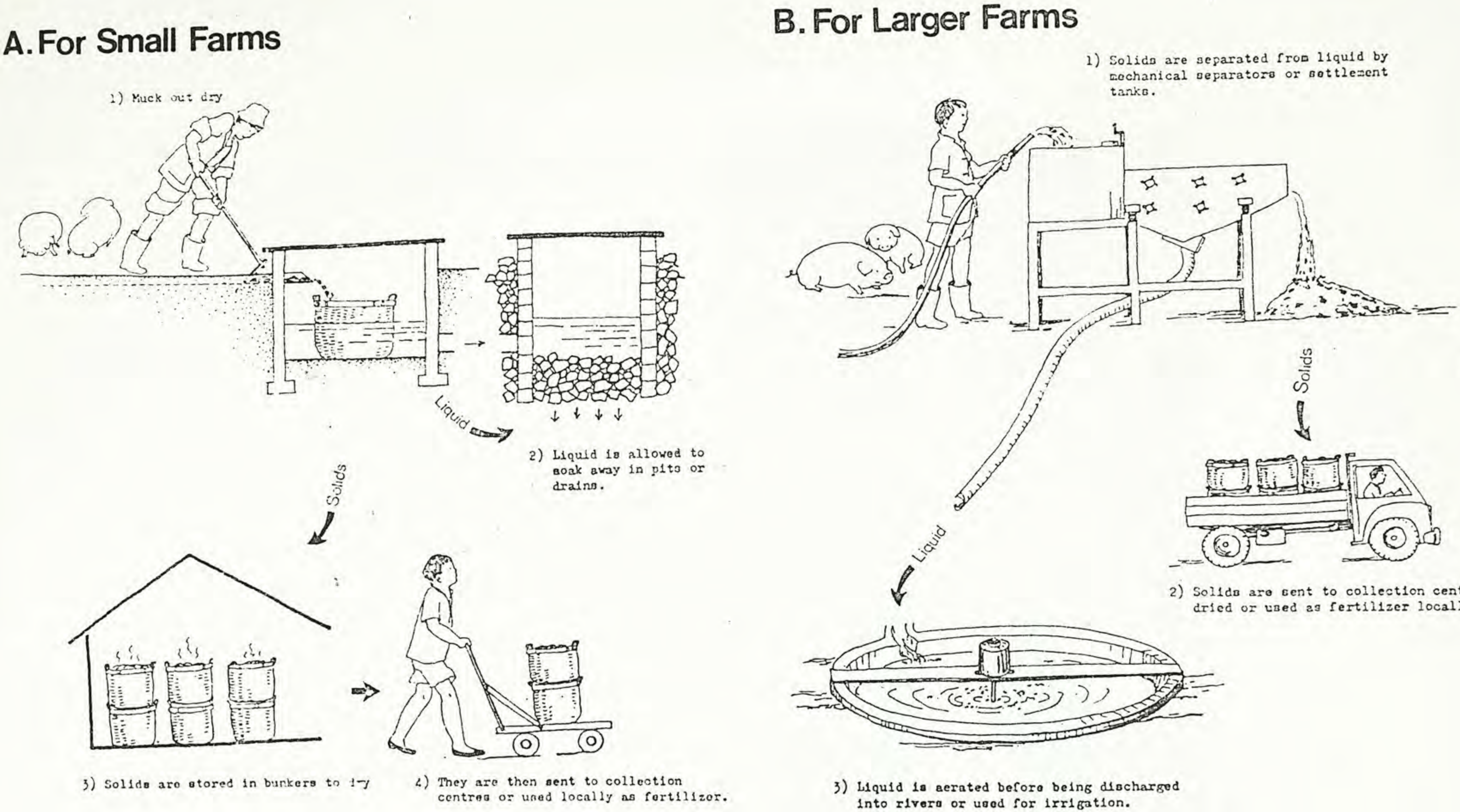


Fig. 1.9 To reduce the problems of agricultural pollution, pig and poultry waste must be treated before discharged into rivers. The principle of the method is to separate the solids from the liquid first, then treat the two separately

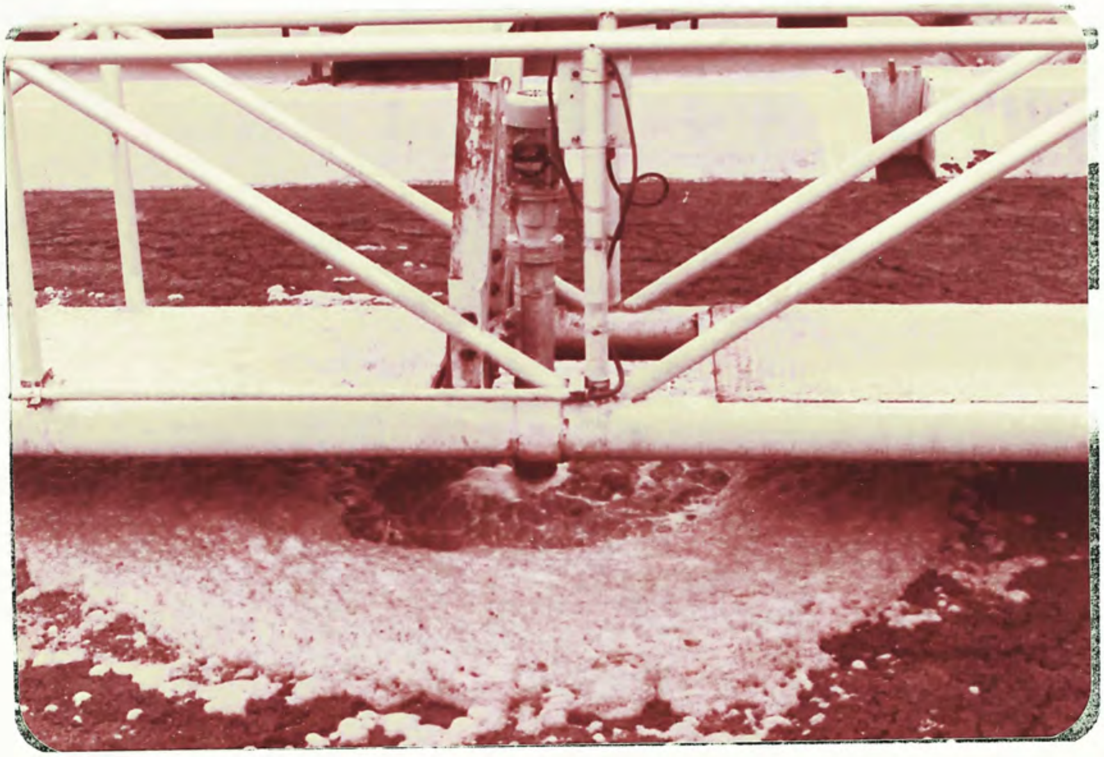

Fig. 1.10 Electric-driven motor, operating 24 hours a day is used to aerate the collected pig farm effluent in the lagoon to facilitate microbial action

Biological filtration tower (Figs. 1.11, 1.12) utilize some inert substances as ground materials for the attachment of micro-decomposers. Effluent after screening is pumped to the top of the tower and let running down through the tower. Running of effluent can be repeated for several times. Filtered effluent can be used for irrigation of edible crops in the farm. The effluent treatment methods used in the experimental farm at Ta Ku Ling of AFD were found to be satisfactory. Preliminary trials using the effluent for irrigating crops showed satisfactory results (Figs. 1.13, 1.14, 1.15).

C Industrial wastes

The Water Pollution Control Ordinance which have been enacted in 1980 will require all liquid effluent dischargers to be licensed. Thus, toxic effluents are expected to be treated before discharge in order to meet the discharge levels specified in licensed conditions. However, existing dischargers who are being exempted from this ordinance may discharge their effluents from their flatted factories. Such effluents may cause damages to the sewage treatment systems which receive them. Another important point is that there are quite a lot of existing factories widely dispersed among our urban areas. Monitoring of such effluents from these factories will need extra work.

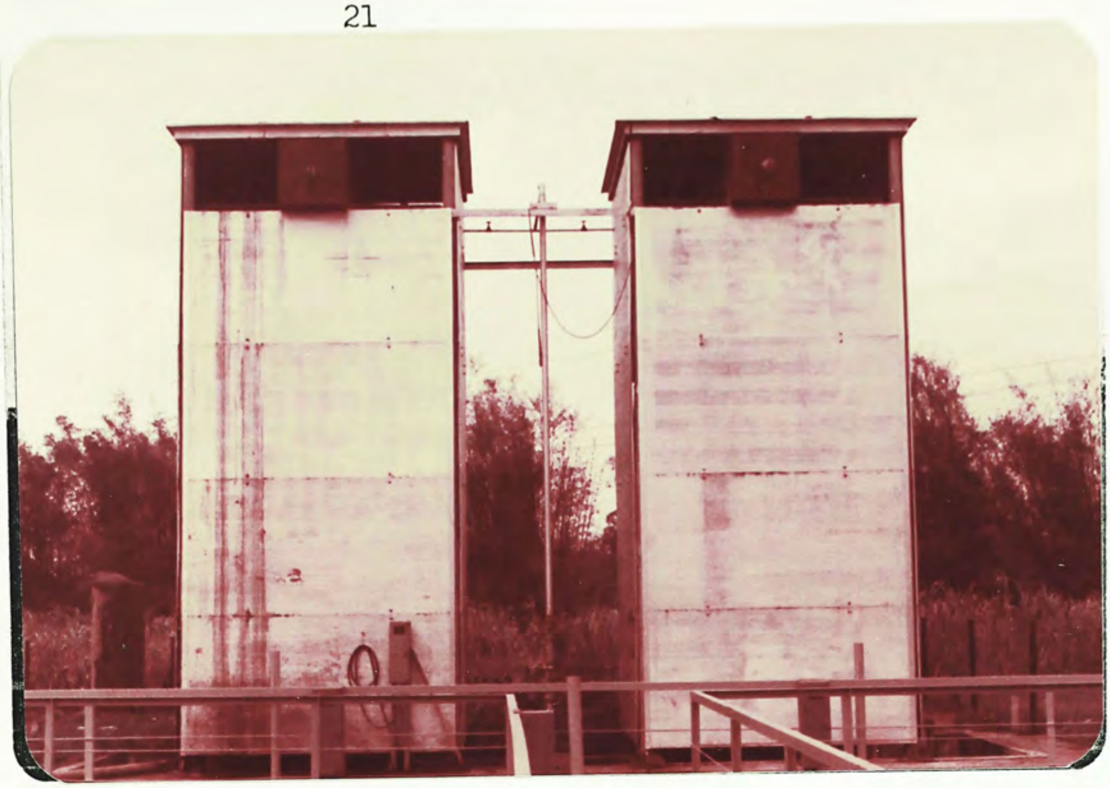

Fig. 1.11 Highrate biological filtration towers used in Ta Ku Ling Station of AFD

Fig. 1.12
The back view of the biological filtration towers. Rock, baskets, bamboo, and plastic pieces are used as substratam for microbial scum attachment

Fig. 1.13 This tanker is used to spray the sludge from the activated lagoon on agricultural land

Fig. 1.14 Sugar canes receive the sludge as fertilizer

Fig. 1.15 Farm effluents after activation treatment is suitable for irrigating edible crops. The Chinese cabbages shown in this photograph have leaves about 60 cm long (Ta Kwu Ling Pilot Treatment Plant AFD)

Having reviewed the present situation of treatment of domestic sewage and animal manure in Hong Kong, we may assume that in the future such wastewater discharges were treated thoroughly in a proper way. However, the sewage sludges and animal manures after the treatment processes will create another disposal problem and may need to be re-utilized in an economical sense. The following section will describe this in a more detail sense.

## 1.4 POSSIBLE WAYS OF DISPOSAL AND RE-UTILIZATION OF SEWAGE SLUDGES AND ANIMAL MANURES

### A Sewage sludges

Sewage sludges can be disposed of in the following ways :

a Disposal into sea by tankers or coastal outfalls

b Disposal as a landfill or tip cover material for reclamation

c Disposal by incineration leaving ash for further disposal

d Disposal by composting resulting in composted product which lacks suitable market outlets.

Sewage sludges can be re-utilized using the following methods :

a <u>Sludge gas production</u>

During the process of anaerobic digestion, bacteria break down the raw sewage in the absence of air and usually at elevated temperature. A by-product, 'Sludge gas' comprising of 67% methane and 33% carbon dioxide. is evolved. Such gas has a calorific value in the range of 580 - 750 BTU/cu.ft. (Coldrick, 1975).

b <u>Direct protein extraction</u>

Direct extraction and recovery of protein from sewage sludges has been found possible. An extraction process, consisting of mechanical homogenization, acidification and thermal treatment, followed by pre-concentration and precipitation was found practicable (Lau, 1980). Evaluation of the nutritional value of the recovered 'sludge protein' indicated that all the essential amino acids were present. Their levels exceeded the amino acid requirements provisionally recommended by the Food and Agriculture Organization (AFO), particularly methionine, cysteine and lysine.

c <u>Algal cultivation</u>

Another possibility is to grow single-celled algae in water extracts of sewage sludges, reducing inorganic substances (ammonia, nitrates and phosphates) and producing an algal biomass rich in protein. Excellent growth of <u>Chlorella pyrenoidosa</u> and <u>Chlorella salina</u> (Wong, 1977; Wong et al., 1977a; Yip and Wong, 1978) and <u>Ulva lactuca</u> (Wong and Lau, 1979) in the sewage sludges extracts has been noted.

d <u>Animal feed supplement</u>

Sewage sludges contain a large number of complex and potentially useful organic molecules (Coldrick, 1975). High levels of protein and Vitamin $B_{12}$ were also found

(Eldred et al., 1976). Thus it is theoretically possible to re-utilize these ingredients by using sewage sludge as animal feed supplement. However, experimental results showed that sewage sludges-supplemented diets were not satisfactory (Wong and Leung, 1979).

Cyprinus carpio, common carp, reared with sewage sludges showed decrease in growth rate, protein and carbohydrate content in body tissue (Wong and Cheung, 1980; Yip and Wong, 1977). Higher metal contents were also found in different parts of the sewage sludge-fed fish (Wong and Kwan, 1981).

e Soil conditioner

The high percentage of organic matter in sewage sludges make it useful as a soil conditioner. Such organic matter is in a stabilized state. The readily decomposed fraction has already been eliminated in the sewage treatment process, especially after anaerobic digestion. Organic matter is believed to improve soil tilth, water infiltration and water holding capacity. Under the name 'Yeo-gro', air dried digested sludge is sold to market gardeners and horticulturalists as a soil conditioner from Penn Mill Recovery Works near Yeovil (Coldrick, 1975).

f Land application

Application of suitable sludge to agricultural land represents a practically valuable re-use of materials. Digested sludge is virtually odourless and inoffensive. It has a more suitable carbon / nitrogen ratio (C/N) for land treatment, and contains a greatly reduced number of pathogenic organisms. To apply sewage sludges on land is a financially attractive way in contrast with other methods of disposal. These materials can improve poor land and, at the same time, helps to solve the disposal problems of densely populated cities. However, one of the new challenges to soil conservationist is to conserve farm land from improper use as a dump for municipal and industrial wastes. Therefore land application of sewage sludges should be carefully monitored such that hazards of contamination is minimized.

B Animal manure

Animal manure can be treated as follows :

a Oxidation ditch

It is a modified form of the activated sludge process where an aeration motor continuously circulates wastewater from farms in an open lagoon. Aerobic microbial action is facilitated. Microbes break down the decomposable part of the wastewater into microbial biomass. Sedimentation afterwards produces effluents with lower nutrient value and sludge suitable for fertilizing use.

b Composting

Composting is a biochemical process of solid organic waste carried out by aerobic, thermophilic micro-organisms. This process is hygienically effective and produce a humus-like material that may be used as a soil conditioner or organic fertilizer.

c Anaerobic digestion

This process utilizes anaerobic microbes to decompose the waste producing digested animal manure and bio-gas with recovery value for biomass and energy. Digested animal manure is more stabilized, odourless and easier to handle.

d Dehydration

Dehydrated animal manure is a usable product to gardeners as fertilizer and farmers as feed supplements. Raw material is mixed with previously dried material to reduce the moisture to less than 40% to facilitate handling. Such a mixture is pulverized in a hammermill and injected into the drier. The product is an odourless, fine, granular material dried to less than 10% moisture.

A large variety of products can be obtained from animal manure. A block diagram illustrating the possibilities of re-utilization was made by Kim and Day (1977) (Fig. 1.16). Re-utilization processes can be categorized into biological, chemical and physical processes.

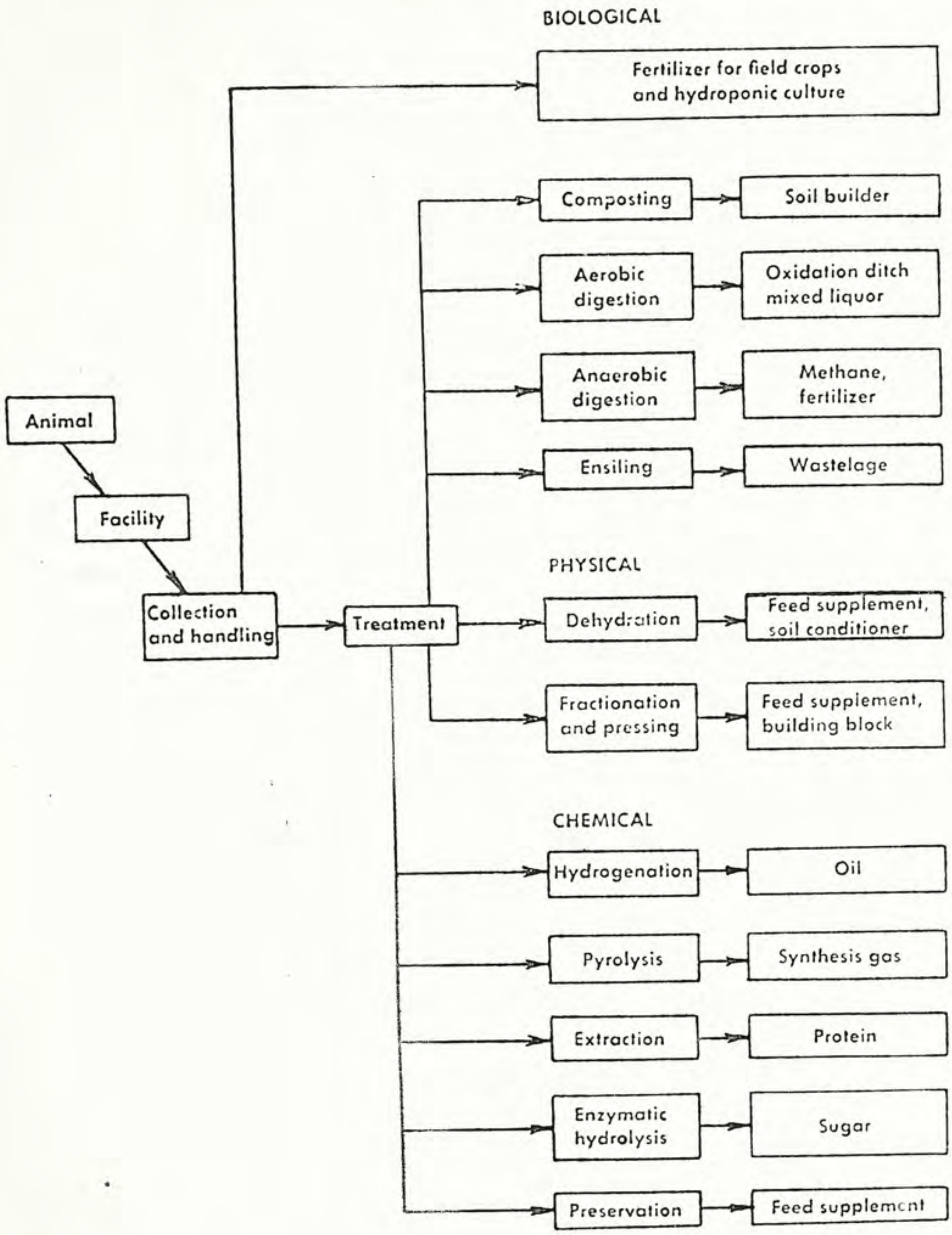


Fig. 1.16 Alternative methods of treating and utilizing livestock wastes (from Kim and Day, 1977)

a Biological processes

i Land application

The application of animal manures to agricultural land by spreading has been a general practice of re-utilization in the past. It serves both as a fertilizer and soil conditioner. Essential inorganic fertilizer components, N, P and K are partially utilized by the crops and some of the organic complex components penetrate into the soil matrix and combine with soil particulates thus improving soil properties.

ii 'Bio-gas' production

Anaerobic digestion of animal manures depends upon the type and viscocity of substrate and the operating conditions. Such operating conditions include temperature, pH and the length of time that the substrate is in the digester. Bio-gas produced contains more than 60% methane and 30% carbon dioxide. For the digestion of one pound of volatile solid about 10 $ft^3$ of bio-gas will be produced which has a calorific value of about 600 BTU/$ft^3$. Besides, digested slurry can be used as fertilizer (Boersma et al., 1978).

iii Nutrient enrichment in aquacultural systems

Single-celled organisms grown in manured pond served as supplementary feed for fish resulted in a large increase in the yield of fish per unit area of pond and sharp decrease in the weight of food which had to be added to produce a kilogram of fish (Schroeder, 1977). *Candida ingens*, a pellicle-forming yeast, was found to grow on substrates derived from anaerobic fermentation of monogastric animal (pig and rat) wastes (Henry et al., 1976). Hydroponic culture, growing water plants on the surface of wastewater, can upgrade the wastewater by filtering some of the chemical pollutants and converting inorganic N and P into organic components, thus reducing the pollution load of receiving watercourses.

iv Animal feed supplement

Poultry manure had been used to replace a portion of diet for feeding various farm animals. Weight gain was found in poultry with additions of poultry manure to 5% in the diet (Ogunmodede and Aninge, 1978). Poultry manure was also found a good source of phosphorus for lambs (Field et al., 1977). Molasses and dried poultry replacing 14% of diet for cattle resulted in equivalent weight gains (Kargaard and Van Niekerk, 1977). Animal manure collected from animal farms was not readily suitable to be used as animal feed. Moisture content and contaminant level were usually not controlled. Production of high-quality manure depended the co-operation of agricultural engineers, animal scientists and veterinarians. Development of integrated systems of feedlot design, manure collection, processing, ingredient mixing, storage and delivery was necessary (Day, 1980).

b Chemical process

i Hydrogenation and pyrolysis

These research methods had been practised in Bureau of Mines U.S.A.. They are based on historical technologies of coal liquefraction and gasification. In the hydrogenation process, the manure is heated under pressure at 380$^{o}$C in the presence of carbon monoxide and steam, for about 20 minutes. The result is a heavy, largely paraffinic oil with a heating value of 14,000 - 16,000 BTU/lb and a sulphur content less than 0.4%. With the pyrolysis method, the manure is heated for about 6 hours in a closed chamber at 900$^{o}$C. At this temperature, the material is converted to gases, such as carbon monoxide, hydrogen and methane. The mixture of gases has a heating value of about 500 BTU/ft$^{3}$. An oil-like material (15,000 BTU/lb) and a solid fraction (5,000 - 13,000 BTU/lb) are also obtained.

ii Direct protein extraction and enzymatic hydrolysis

Using 1 N sodium hydroxide as an extractant, as much as 340 mg of protein can be extracted from 1 gm of manure on a dry basis. Using strong oxidants, up to 230 mg of glucose equivalent of reducing sugars can be obtained. These sorts of chemical re-utilization methods were carried out in Colorado State University (Kim and Day, 1977).

iii Chemical preservation

Low level of formalin is added to the fresh manure in the research study at the University of Illinois to control pathogens and odours, increase palatability and preserve the nutritive value. Other feed ingredients are then added to formulate a balanced diet to refeed the animal (Kim and Day, 1977).

c Physical process

Dehydration has been a common practice to handle the animal manure. The dried product can be utilized as a soil conditioner or as a feed supplement.

## 1.5 AIM OF THE WHOLE THESIS

The aim of this thesis is two-folded. One purpose is to search for the possible re-utilization ways of sewage sludges (activated sludge and digested sludge) and animal manures (chicken manure and pig manure) for biomass production via alga, vegetable and fish accordingly. Part One of this thesis was designed for this purpose : A comparative experimental study on the properties of the four wastes, including their physical properties, nutrient levels and metal contents was presented in Chapter Two. Chapter Three, Four and Five described the results of three experimental studies on the utilization of the four wastes for the cultivation of a green unicellular alga (Chlorella pyrenoidosa), common carp (Cyprinus carpio) and Flowering Chinese Cabbage (Brassica parachinensis).

The second purpose of this thesis is to study the subsequent effects of utilizing these four wastes on organism of higher trophic levels in two simulated food chains. In Chapter Six, the metal contents (Pb, Cu, Zn, Mn) in different trophic levels of a simulated aquatic food chain (waste → unicellular green alga, Chlorella pyrenoidosa → freshwater shrimp, Palaeomonetes sp.) were analysed. Another simulated terrestrial food chain (waste → Flowering Chinese Cabbage, Brassica parachinensis and Chinese Radish, Rhaphanus sativus → Common White Butterfly, Pieris canidia) study was also conducted (Chapter Seven). The general conclusion of the whole thesis was given in Chapter Eight.

# CHAPTER TWO

# Properties of sewage sludges and animal manures

## 2.1 INTRODUCTION

### A Properties of sewage sludges

Sewage sludge is a liquid-solid mixture containing contaminants removed from wastewater by physical, biological and chemical treatments at the sewage treatment plants. With the increasing knowledge on sewage treatment process, the treated effluents have high water quality but the levels of contaminants in the sewage sludge will be raised.

The chemical composition of sewage sludges is dependent on numerous factors, including the treatment process, the extent and nature of industrialization in the sanitary district and the seasonal variability of sewage entering the treatment plant (Wong and Yip, 1980).

Concerning with the treatment process, sewage treatment plants produce sewage sludges of three kinds during different stages of treatment :

a Primary treatment is simply an initial settling out of solids yielding a raw sludge, high in nitrogen and relatively undecomposed.

b Secondary treatment is an aerated, rapid microbial digestion that converts much of the raw organic matter into microbial biomass. Microbes are usually killed after the process.

c Effluent contains soluble salts, e.g. N, P, K, Na and B. <u>Tertiary treatment</u> is the further removal of nutrients and suspended soilds in the effluent after secondary treatment so as to reduce the risk of eutrophication to the receiving waters.

A regional survey of composition of sewage sludge was conducted by obtaining data for 30 constituents in more than 250 samples from approximately 150 treatment plants. Computation of the mean and median values indicated that N, P and K levels were within a relatively narrow range. Whereas these statistics demonstrated that lead (Pb), copper (Cu), zinc (Zn), nickel (Ni) and cadmium (Cd) concentrations were extremely variable (Table 2.1) (Sommers, 1977). The same sort of results were also demonstrated by Menzies (1973).

Thus, sewage sludges when considered as fertilizer had to be rated low. The mean N, P and K value found by Menzies (1973) basis was 2 - 1 - 0.2% compared with that of chicken manure (6.7 - 14.4 - 2%) and pig manure (7.7 - 25 - 5%) (Hills, 1975; Loehr, 1974; Midwest Plan Service, New Zealand, 1975). However, the heavy metals in sewage sludges are of most importance. They include Pb, Cu, Zn, manganese (Mn), Ni and Cd. These are potentially toxic to living organisms and may be hazardous in the food chains. Some metals (e.g. Cd and Ni either may accumulate in the edible portion of agronomic crops or may cause phytotoxicity (Berrow and Webber, 1972; Chaney, 1973).

Table 2.1 Chemical composition of sewage sludge reported by Sommers (1977) and Menzies (1973)

| | Sommers, 1977 | Menzies, 1973 |
|---|---|---|
| N | 0.50 - 17.60 % | 1.0 - 6.0 % |
| P | 0.50 - 14.30 % | 1.0 - 3.0 % |
| K | 0.02 - 2.64 % | 0.2 - 0.5 % |
| Pb | 464 - 11,897 ppm | 100 - 10,000 ppm |
| Cu | 452 - 11,875 ppm | 250 - 17,000 ppm |
| Zn | 819 - 28,850 ppm | 500 - 50,000 ppm |
| Ni | 40 - 4,016 ppm | 25 - 8,000 ppm |
| Cd | 3 - 1,177 ppm | 5 - 2,000 ppm |

B Properties of animal manures

A summary of results on characteristics of chicken manure and pig manure reported by various authors (Hills, 1975; Loehr, 1974; Middlebrooks, 1974 and Midwest Plan Service, New Zealand, 1975) was compiled in Table 2.2 in order to compare their differences.

BOD (biochemical oxygen demand) determination is an empirical test to determine the relative oxygen requirement of wastewaters, effluents and polluted waters (American Public Health Association, 1976). COD (chemical oxygen demand) determination is a measure of the oxygen equivalent of that portion of the organic matter in a sample that is susceptable to oxidation by a strong chemical oxidant (American Public Health Association, 1976).

The production of raw manure per weight of animal was essentially the same for all domestic animals. However, other characteristics show a wider range because of the difference in moisture content. Pig manure had a relatively higher moisture content (90.91%) than that of chicken manure (74.65%). This account for the difference in the percentage of total solids between chicken manure (25.35%) and pig manure (9.09%).

Percentage of volatile solids / total solids was also higher in pig manure (80.0%) than chicken manure (66.7%). Components of malodor volatile substances of pig manure had been studied by Yasuhara and Fuwa (1977a, 1977b, 1978, 1979a,

Table 2.2 Characteristics of chicken manure and pig manure (Hills, 1975; Loehr, 1974; Middlebrooks, 1974; Midwest Plan Service, 1975)

| | Chicken Manure | Pig Manure |
|---|---|---|
| | (values in gm/kg body weight of animal/day) | |
| Raw manure | 71.00 | 66.00 |
| $BOD_5$ | 3.46 | 3.10 |
| $COD_5$ | 9.80 | 6.40 |

| | Percentage (base on) | | Percentage (base on) | |
|---|---|---|---|---|
| | Raw Manure | Total Solids | Raw Manure | Total Solids |
| Total solids | 25.35 | 100.00 | 9.09 | 100.00 |
| Volatile solids | 16.09 | 66.70 | 7.27 | 80.00 |
| Total nitrogen | 1.69 | 6.67 | 0.70 | 7.67 |
| Total phosphorous | 3.66 | 14.44 | 2.27 | 25.00 |
| Total potassium | 0.51 | 2.00 | 0.54 | 5.00 |

1979b, 1979c) by using gas chromatography and mass spectrophotometer. Such compounds included sulphur-compounds (hydrogen sulfide and dimethyl sulfide), carboxylic acids (butyric, isovaleric, and phenylacetic acids), phenols (o-, m- and p-cresols) and neutral nitrogen-containing substances (indole, skatole and o-aminoacetophenone).

Concerning with the fertilizing ability of the animal manures, N, P and K value of chicken manure (1.7 - 3.7 - 0.5%) was comparatively higher than that of pig manure (0.7 -2.3 - 0.5%) (Table 2.2). Similar results were found on BOD (chicken manure 3.5, pig manure 3.1 gm/kg body weight/day) and COD (chicken manure 9.8, pig manure 6.4 gm/kg body weight/day) values (Table 2.2).

The purpose of this experimental analysis on properties of sewage sludge and animal manure was to investigate the differences among these wastes in the sense of physical, organic and nutrient characteristics. Besides, heavy metal contents of these wastes was also analysed so as to compare their suitability for re-utilization.

## 2.2 MATERIALS AND METHODS

### A Collection and treatment of samples of sludge and manure

The sludge samples were obtained from two sites : activated sludge from the Sewage Treatment Plant at the Chinese University of Hong Kong; digested sludge from Shek Wu Hui Sewage Treatment Plant. The manure samples including chicken manure and pig manure were collected from farms in the New Territories of Hong Kong. They were dried under the sun for one week and grinded by means of a hand-driven grinder. Both sludge samples and manure samples were sieved through a 2 mm sieve before use.

B Analysis of the properties of the wastes

The following methods were used : total nitrogen (Kjeldahl, 1883), extractable phosphate (extractant : 2.5% of acetic acid, molybdenum blue method, Watanabe and Oslen, 1962), organic carbon (Walkey and Black, 1934), ash (muffle furnace, $500^{o}C$) and moisture (oven, $105^{o}C$). Total heavy metals (mixed acid digestion) and exchangeable heavy metals (extractant : 1 M ammonium acetate solution at pH = 7) were analysed using an atomic absorption spectrophotometer following the method described by Allen et al. (1974).

## 2.3 RESULTS

Activated sludge has the lowest content of total nitrogen, extractable phosphate and organic carbon and the highest ash content among the four wastes. Digested sludge has similar contents of total nitrogen and extractable phosphate when compared with chicken manure and pig manure, but a lower level of organic carbon and a higher level of ash. In moisture content no significant difference ($p > 0.05$) has observed between the four wastes except that digested sludge has a comparatively lower moisture content (Table 2.3).

Table 2.3 Properties of animal manures and sewage sludges in the present study

| | Activated Sludge | Digested Sludge | Chicken Manure | Pig Manure |
|---|---|---|---|---|
| Total Nitrogen Content % | 0.588±0.301 | 2.085±0.040 | 2.056±0.081 | 2.524±0.040 |
| Extractable Phosphate Content ppm | 28.33±10.59 | 292.5±36.23 | 261.3±54.24 | 344.2±46.95 |
| Organic Carbon Content % | 5.95±0.73 | 9.83±3.10 | 32.84±0.21 | 48.24±1.29 |
| Ash Content % | 83.45 | 74.76 | 39.06 | 20.24 |
| Moisture Content % | 11.54 | 4.59 | 13.87 | 12.06 |

Table 2.4 compares the total contents and the exchangeable contents of Pb, Cu, Zn and Mn in the four wastes. The total contents of the metals do not correlate with their exchangeable contents. This is especially true in the case of Zn where activated sludge has the lowest total content (149 ppm) but the highest exchangeable content (139 ppm) among all wastes.

Considering the total contents, digested sludge has the highest Pb, Cu and Zn levels whereas chicken manure has the highest level of Mn. In exchangeable contents all the wastes are similar except that activated sludge and digested sludge have a very high content of Zn and activated sludge the lowest level of Mn.

## 2.4 DISCUSSION

When the fertilizing ability of the four wastes were compared, it was found that activated sludge seemed to be the least favourite among the four if they were used as fertilizers. Its total nitrogen content and extractable phosphate content were around one-fourth and one-tenth of that of the other three wastes respectively (Table 2.3). As to the organic carbon content, two types of sewage sludge had values below 10% (5.95% for activated sludge and 9.83% for digested sludge). These values were relatively low when compared with animal manures (32.85% for chicken manure amd 48.24% for pig manure). Menzies (1973) claimed that this decrepancy in organic matter was due to sewage sludges contain

Table 2.4 Heavy metal contents of animal manure and sewage sludges (ppm), each value was a mean ± standard deviation

| | Activated Sludge | Digested Sludge | Chicken Manure | Pig Manure |
|---|---|---|---|---|
| Lead | | | | |
| Total | 20.00± 8.94 | 33.33± 5.16 | 20.00± 6.32 | 8.33± 2.58 |
| Exchangeable | 8.33± 2.04 | 9.17± 2.04 | 8.57± 1.37 | 9.58± 1.88 |
| Copper | | | | |
| Total | 52.50± 7.75 | 105.00± 17.54 | 85.00± 15.73 | 52.50±12.25 |
| Exchangeable | 15.40± 4.35 | 14.85± 5.63 | 18.13± 1.94 | 14.15± 3.28 |
| Zinc | | | | |
| Total | 149.17±24.98 | 753.33±157.18 | 230.00±20.74 | 372.50±38.83 |
| Exchangeable | 139.38± 9.28 | 86.88± 4.59 | 8.13± 0.68 | 7.50± 1.77 |
| Manganese | | | | |
| Total | 23.33± 8.16 | 173.33± 26.58 | 390.00±38.86 | 195.00±24.29 |
| Exchangeable | 3.75± 2.50 | 26.46± 1.23 | 20.83± 3.03 | 15.83± 8.61 |

a high content of silt, sand and other inorganic wastes (from 30 to 60%). Evidence was also found in the difference in ash content among the four wastes (83.45% in activated sludge, 74.76% in digested sludge, 39.06% in chicken manure and 20.24% in pig manure).

Some calculations were made on the metal content of the four wastes (Table 2.5). The sum of the mean values of Pb, Cu, Zn and Mn was found. By dividing the sum of total metal content, percentage of exchangeable / total was also evaluated.

Digested sludge had the highest level of the sum of the total metal contents (1,065 ppm) while activated sludge had the lowest level (245 ppm). Chicken manure and pig manure had levels in between the two extremes (725 ppm and 628 ppm respectively).

When considering the sum of exchangeable metal contents, both activated sludge and digested sludge had levels (167 ppm and 137 ppm respectively) higher than that of both chicken manure and pig manure (56 ppm and 47 ppm respectively). Generally speaking, sewage sludges had exchangeable metal contents about three times as much as the animal manures. Such results correlated with that of Menzies (1973) who stated that sewage sludge contain a great variety of metal elements and in amounts much higher than the trace levels in animal manures.

Table 2.5 Mathematical analysis on the data of metal content of animal manures and sewage sludges

| | Activated Sludge | Digested Sludge | Chicken Manure | Pig Manure |
|---|---|---|---|---|
| Sum of total metal content (ppm) | 245.00 | 1064.99 | 725.00 | 628.33 |
| Sum of exchangeable metal content (ppm) | 166.86 | 137.36 | 55.66 | 47.06 |
| Percentage of $\frac{\text{exchangeable}}{\text{total}}$ | 68.11 | 12.90 | 7.68 | 7.49 |

The reason why activated sludge, which had the lowest sum total metal content, also possessed sum exchangeable metal content comparable with digested sludge. Activated sludge had an extraordinary high value of 68% of exchangeable / total metal content when compared with digested sludge (13%). This implied that the availability of metals from the wastes. were different in different wastes. On the other hand, low percentages of exchangeable / total metal content were found in the animal manures (around 7.5%).

Examination of the properties of the four wastes in this experiment revealed the differences in their physical properties, nutrient levels and metal content. However, only physical and chemical examinations do not provide sufficient information on their effects on living organisms. In order to investigate the effects of such wastes on biota. Experiments related to algal culture, culture of fish and culture of vegetable have been carried out. Results of the experiments are presented in the following chapters of Part One (Chapter 3, 4 and 5).

# CHAPTER THREE

## Utilization of activated sludge, digested sludge, chicken manure and pig manure for the cultivation of Chlorella pyrenoidosa, a unicellular green alga

### 3.1 INTRODUCTION

The use of algae to convert waste materials to a usable feed grade protein supplement has received the most attention and appears to have the most promising future. Chlorella has been studied largely because it can be sychronized easily with light-dark cycle. Interest has been renewed in producing single-celled protein by mass culture of unicellular algae (Goldman and Ryther, 1975). Algal systems can be used for both pollution control and protein production by directly recycling the nutrients in wastewaters into biomass.

Recently, it has been noted that aqueous extracts of sewage sludges supported excellent growth of Chlorella pyrenoidosa and Chlorella salina (Wong, 1977; Wong and Ho, 1977; Wong et al., 1977a; Yip, 1976; Yip and Wong, 1978) and Ulva lactuca (Wong and Lau, 1979).

The purpose of this experiment described in this chapter is to compare the suitability of four wastes : activated sludge, digested sludge, chicken manure and pig manure for the cultivation of Chlorella pyrenoidosa, a unicellular green alga.

## 3.2 MATERIALS AND METHODS

A Preparation of media for cultivation of algae :

Concentrations of the media were based on a weight to weight base. For sludge samples, 6% w/w solutions were prepared. For manure samples, 2% w/w was used. Extraction of the sludge or manure was by means of a 20 minutes autoclaving method followed by suction filtration through Whatman No. 42 filter papers. Filtered sludge and manure extracts were used as stock solutions and suitably diluted to give 0.5, 1, 2, 4 and 6% solutions for sludge-extract media and 0.1, 0.2, 0.5, 1 and 2% solutions for manure-extract media. Bristol medium was used as a control.

B Analysis of properties of waste extracts :

The pH and heavy metal contents of the various extracts were measured.

C Cultivation of algae in various media :

A volume of 75 ml for each medium was put into a 250 ml conical flask and autoclaved again for 20 minutes. A duplicate set of solutions was made. 1 ml of Chlorella pyrenoidosa suspension (concentration = $3.07 \times 10^7$ cell per ml) was innoculated into each container. The flasks were then placed on the illuminated benches for 11 days at $25^oC \pm 2^oC$ temperature and 70 80% relative humidity with a 16 hour light (2,500 lux) / 8 hour dark cycle. The flasks were shaken twice daily in order to aerate the media of growth (Fig. 3.1).

Fig. 3.1 Illuminated bench with automatic timer control (16 hour light / 8 hour dark cycle) used for the experiment.

D Analysis of properties of waste grown algae :

The number of algal cells was recorded every other day for a culture period of 11 days using a Neübauer counter chamber. Oven-dried weight (oven, 105°C), chlorophyll content (Arnon, 1949) and heavy metal content (dry ashing method, Allen et al., 1974) of the algal products were tested at the end of the culture period.

## 3.3 RESULTS

A Properties of the waste extracts :

Table 3.1 shows the pH values and the values of heavy metals in the aqueous extracts of the waste materials compared with the Bristol medium for the cultivation of Chlorella pyrenoidosa. Activated sludge-extract medium has the lowest pH (4.5) and this is rather acidic for algal growth. Digested sludge-extract medium has a pH similar to that of Bristol medium (6.5) whereas chicken manure-extract medium and pig manure-extract medium are more alkaline (about 7.2). Aqueous extracts of activated sludge and digested sludge have higher levels of some heavy metals than the manure-extract and Bristol medium, except chicken manure which has the highest content of Cu. Activated sludge has a significantly higher level of Zn and digested sludge of Mn. The contents of these metals, together with their pH values must be reflected in suitabilities as media for algal growth.

Table 3.1 Values of pH and contents of heavy metals in various extracts of wastes used for the cultivation of Chlorella pyrenoidosa compared with Bristol medium

| | Activated Sludge 6% | Digested Sludge 6% | Chicken Manure 2% | Pig Manure 2% | Bristol Medium |
|---|---|---|---|---|---|
| pH | 4.50 | 6.30 | 7.18 | 7.15 | 6.47 |
| Lead (ppm) | 0.20 | 0.30 | 0.05 | 0.05 | 0.05 |
| Copper (ppm) | 0.63 | 0.63 | 0.78 | 0.46 | 0.48 |
| Zinc (ppm) | 6.20 | 0.70 | 0.45 | 0.55 | 0.05 |
| Manganese (ppm) | 0.15 | 1.00 | 0.35 | 0.20 | 0.18 |

B Growth rates of algae in various waste extracts :

a <u>Activated sludge extracts</u> (Fig. 3.2) :

Bristol medium provides a better growth for the algae than any activated sludge extracts. Extracts of lower concentrations (0.5, 1 and 2%) support an increase in cell number whilst those of higher concnetrations (4 and 6%) do not support any increase in cell number during the cultivation period.

b <u>Digested sludge extracts</u> (Fig. 3.3) :

The growth curves of the algae in digested sludge extracts fluctuate due to unknown reasons. However, the results show that the digested sludge extracts provide better growth than the Bristol medium. Extracts of 1, 2 and 4% show better growth conditions than that in extracts of 0.5 and 6%.

c <u>Chicken manure extracts</u> (Fig. 3.4) :

This set of growth curves shows that extracts of low concentrations (0.1 and 0.2%) provide better growth for the algae than extracts of higher concentrations, 0.2% extract provides the best growth.

d <u>Pig manure extracts</u> (Fig. 3.5) :

Extracts of 0.2, 0.5 and 1% provide better growth for the algae than 0.1 and 2% extracts. 0.5% extract has the highest growth rate among all concentrations tested.

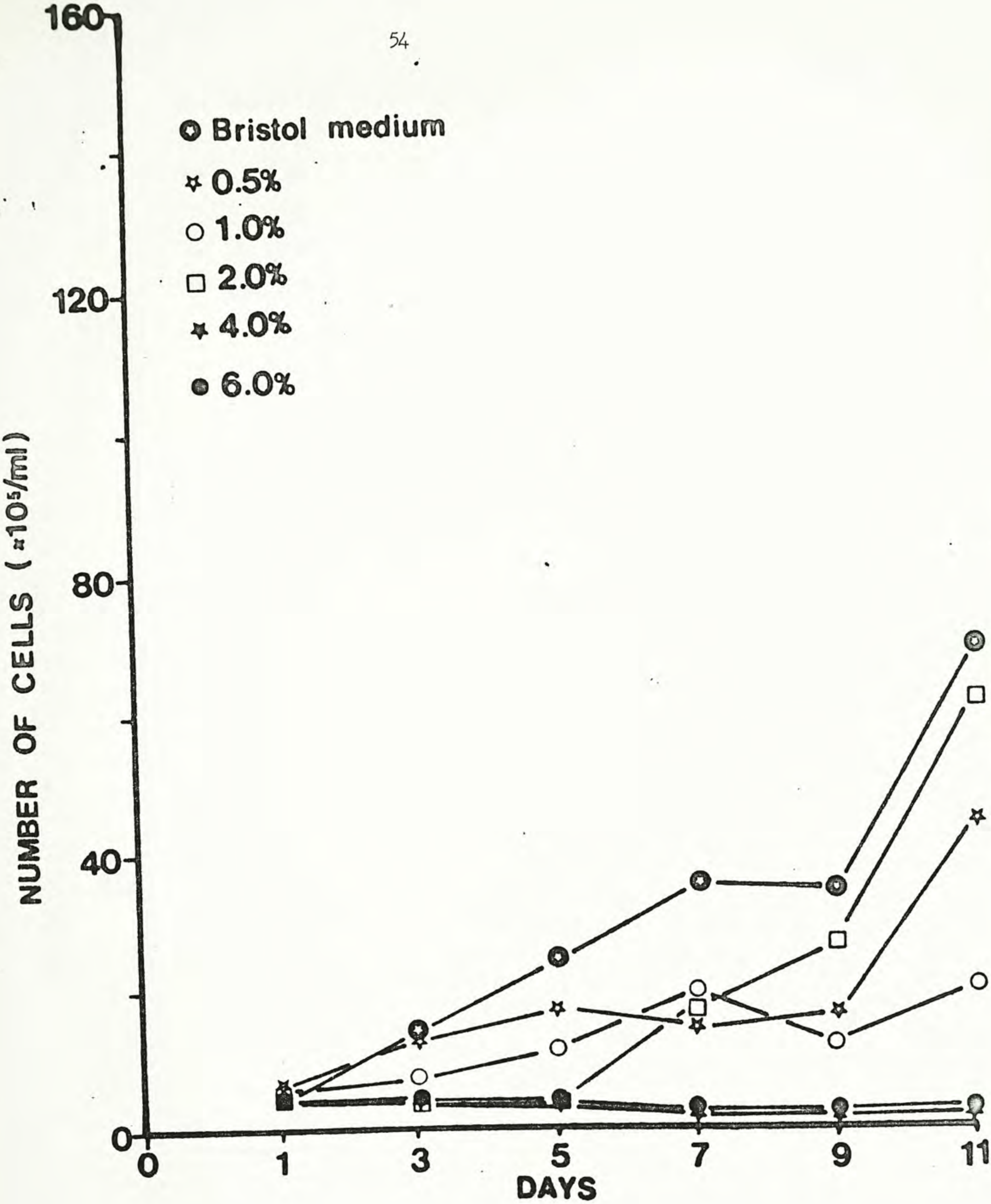


Fig. 3.2 Growth curves of Chlorella pyrenoidosa in different concentrations of activated sludge extracts

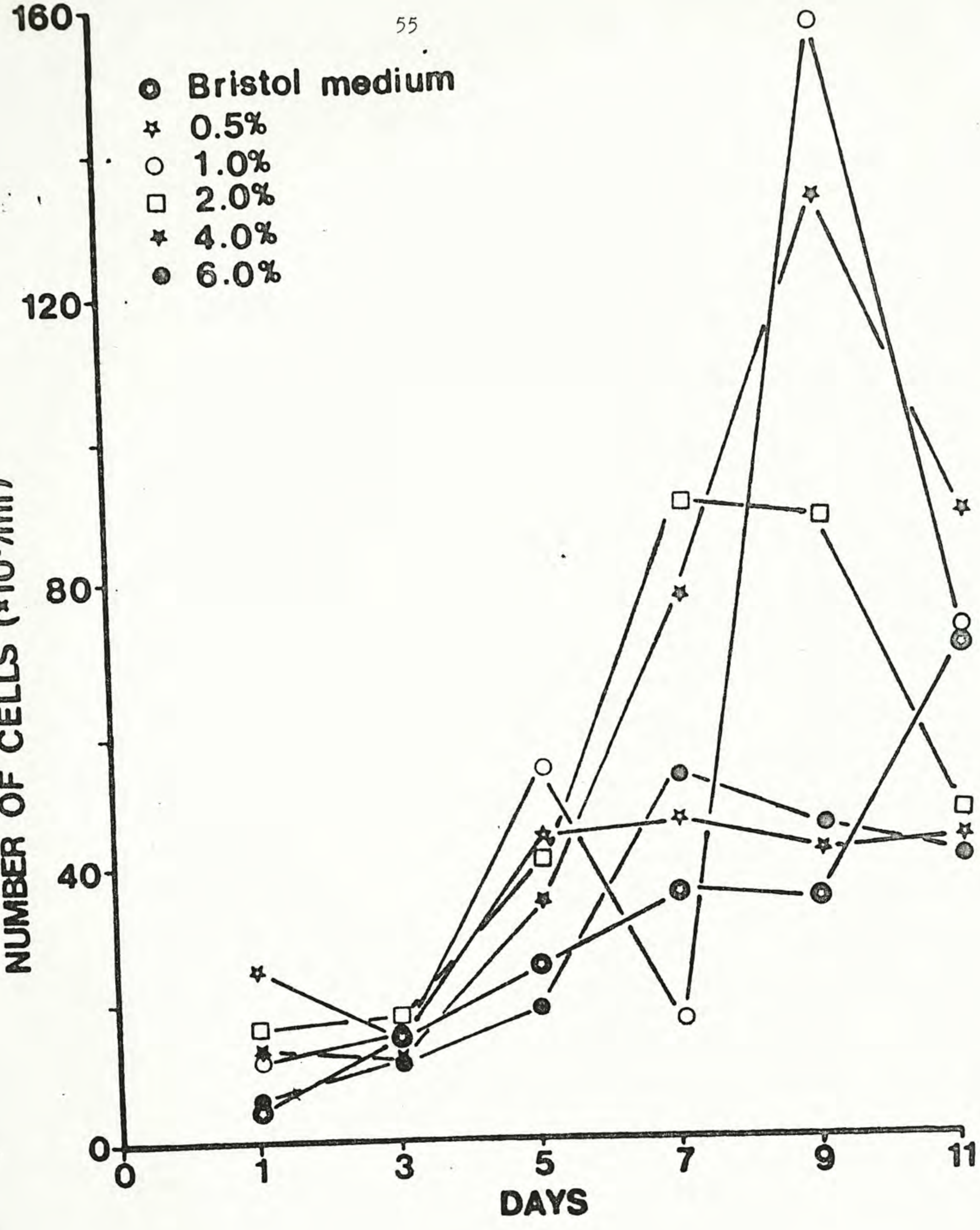


Fig. 3.3 Growth curves of Chlorella pyrenoidosa in different concentrations of digested sludge extracts

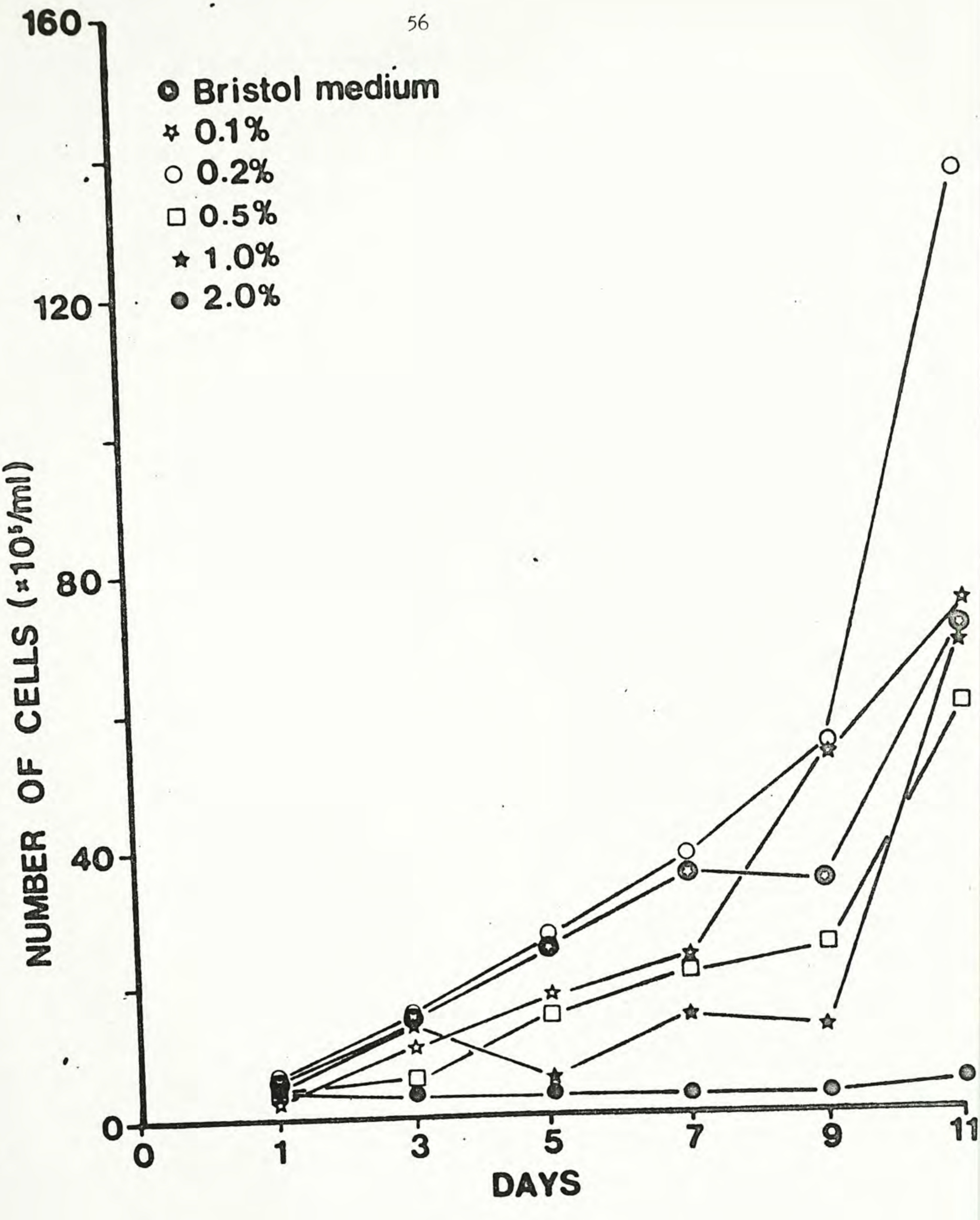


Fig. 3.4 Growth curves of Chlorella pyrenoidosa in different concentrations of chicken manure extracts

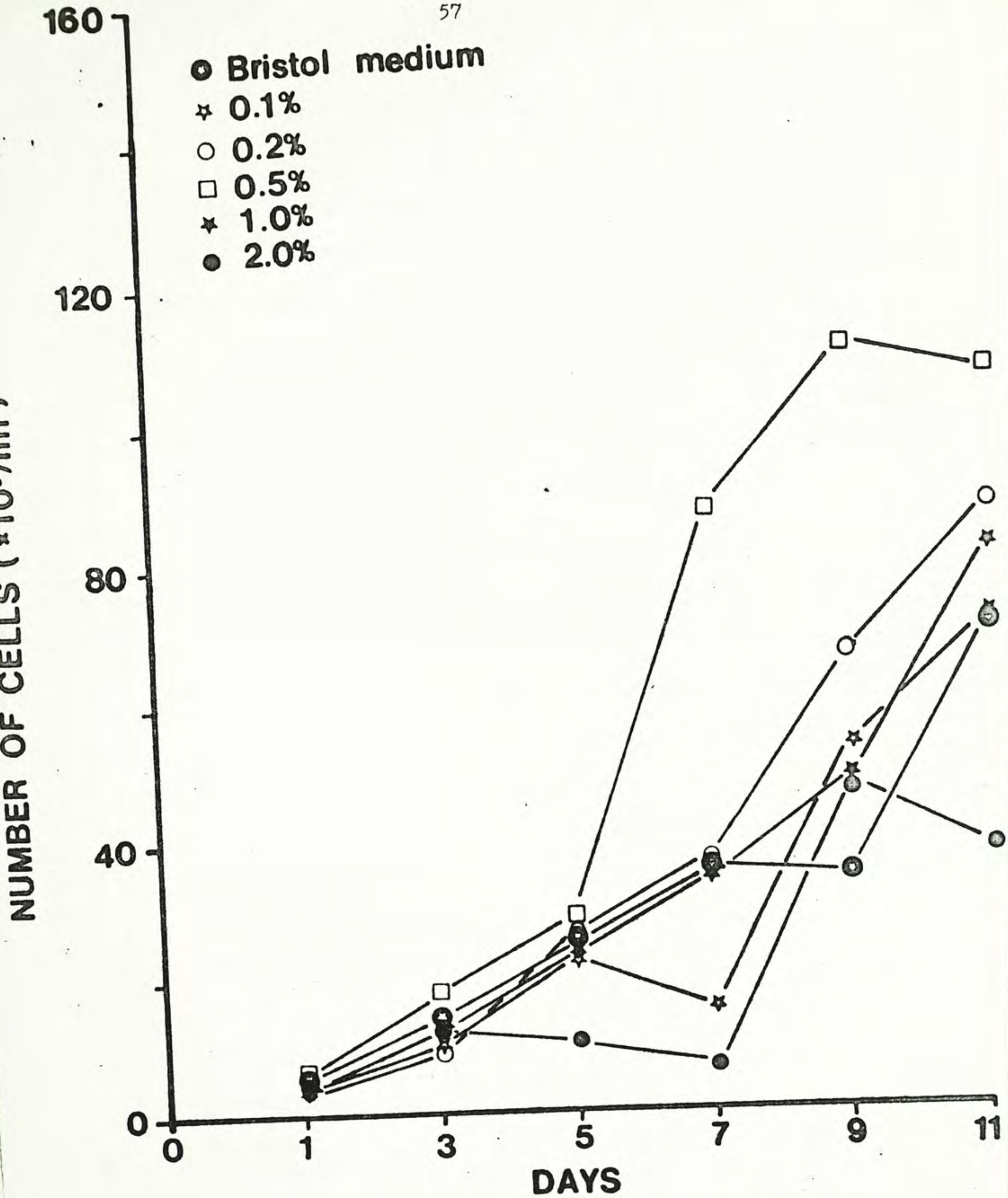


Fig. 3.5 Growth curves of Chlorella pyrenoidosa in different concentrations of pig manure extracts

Out of the four wastes used in this experiment, it is found that extracts of activated sludge do not provide better algal growth than Bristol medium. For the other three wastes : digested sludge, chicken manure and pig manure, at least some of the selected concentrations supported better algal growth than the Bristol medium. These media include 1, 2 and 4% digested sludge-extracts; 0.1 and 0.2% chicken manure-extracts; 0.2, 0.5 and 1% pig manure extracts.

C Oven-dried weight of harvested algae (Fig. 3.6) :

Activated sludge extracts produce the lowest oven-dried weight of the harvested algae (range : 5.3 - 12.7 mg per 100 ml). Extracts of other wastes produce higher oven-dried weight of the harvested algae (range : 11.6 - 57.0 mg per 100 ml for digested sludge extracts; 14.5 - 60.8 mg per 100 ml for pig manure extracts). However, the oven-dried weight of harvested algae was not always correlated to the cell number of the algae obtained at the end of the culture period, especially with extracts of higher concentrations. This may be due to the difference in the size of algal cells grown in different media. On the other hand, oven-dried weight determination may also include cell debris in the culture media.

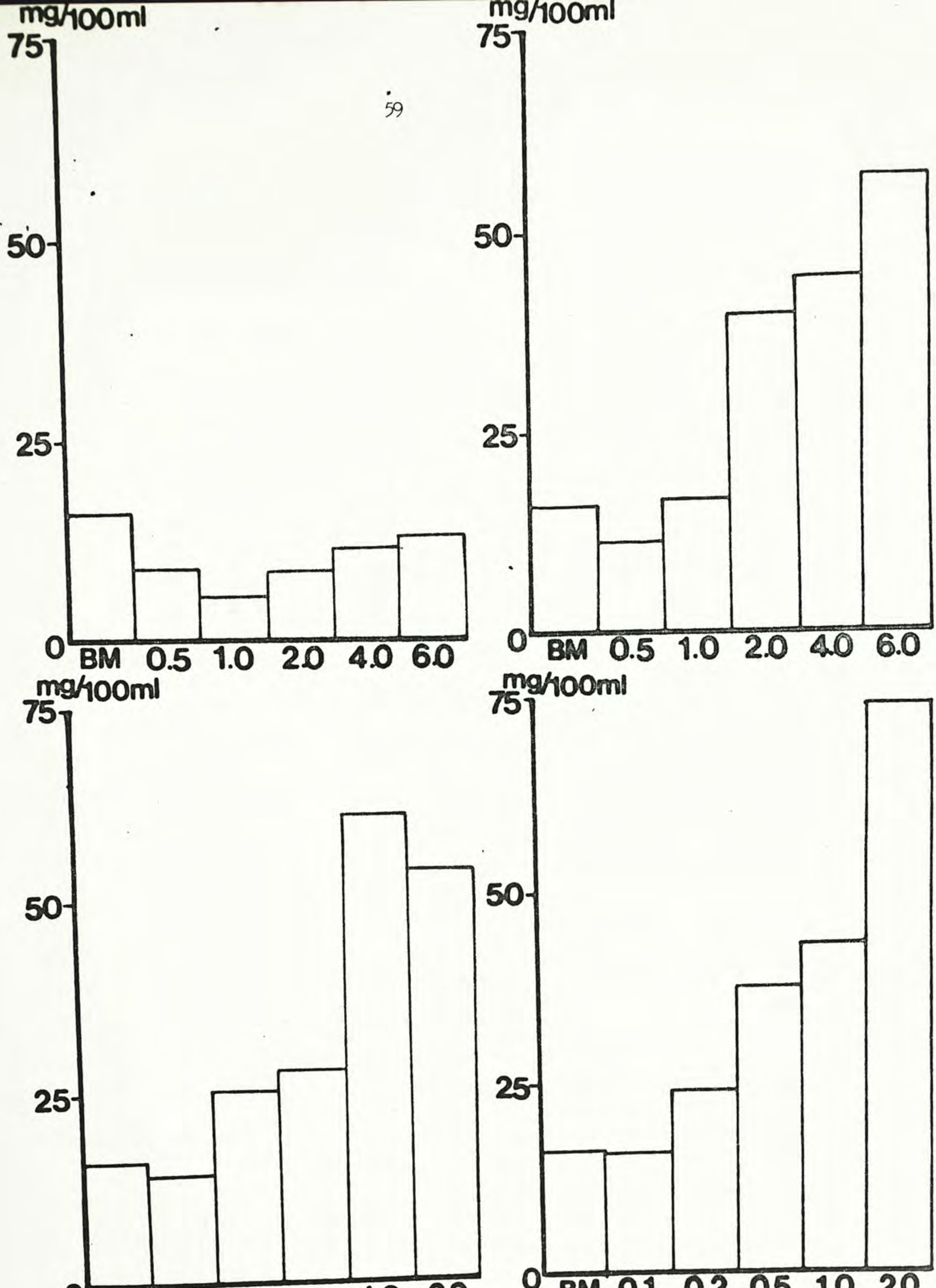


Fig. 3.6 Comparison of oven-dried weight (mg/100ml) of harvested <u>Chlorella</u> <u>pyrenoidosa</u> from different culture media

D Chlorophyll content in harvested algae (Fig. 3.7) :

Chlorophyll contents in cell suspensions after culture correlate with the oven-dried weight of the harvested algae in the suspension. Extracts of activated sludge show lower chlorophyll content (range : 0.84 - 3.62 µg per ml) whereas extracts of the other three wastes show higher chlorophyll content after culture (range : 8.26 - 29.95 µg per ml for digested sludge extracts; 5.12 - 26.38 µg per ml for chicken manure extracts; 5.53 - 36.71 µg per ml for pig manure extracts).

E Heavy metal contents of harvested algae :

Levels of Pb, Cu, Zn and Mn in harvested algae are illustrated in Table 3.2. It was found that the levels of heavy metals in harvested algae were negatively correlated with oven-dried weights and chlorophyll contents of the media after culture. High concentrations of heavy metals were found in algae harvested from activated sludge extracts, whilst extracts of chicken manure and pig manure produced algae with lower metal concentrations.

The highest concentrations of heavy metals in the harvested algal cells are found in 1% activated sludge extract (Pb 743.4 ppm, Cu 3,655.7 ppm, Zn 6,603.8 ppm and Mn 707.5 ppm). This may be due to the rather rapid algal growth in the 1% extract with the rapid uptake of various heavy metals.

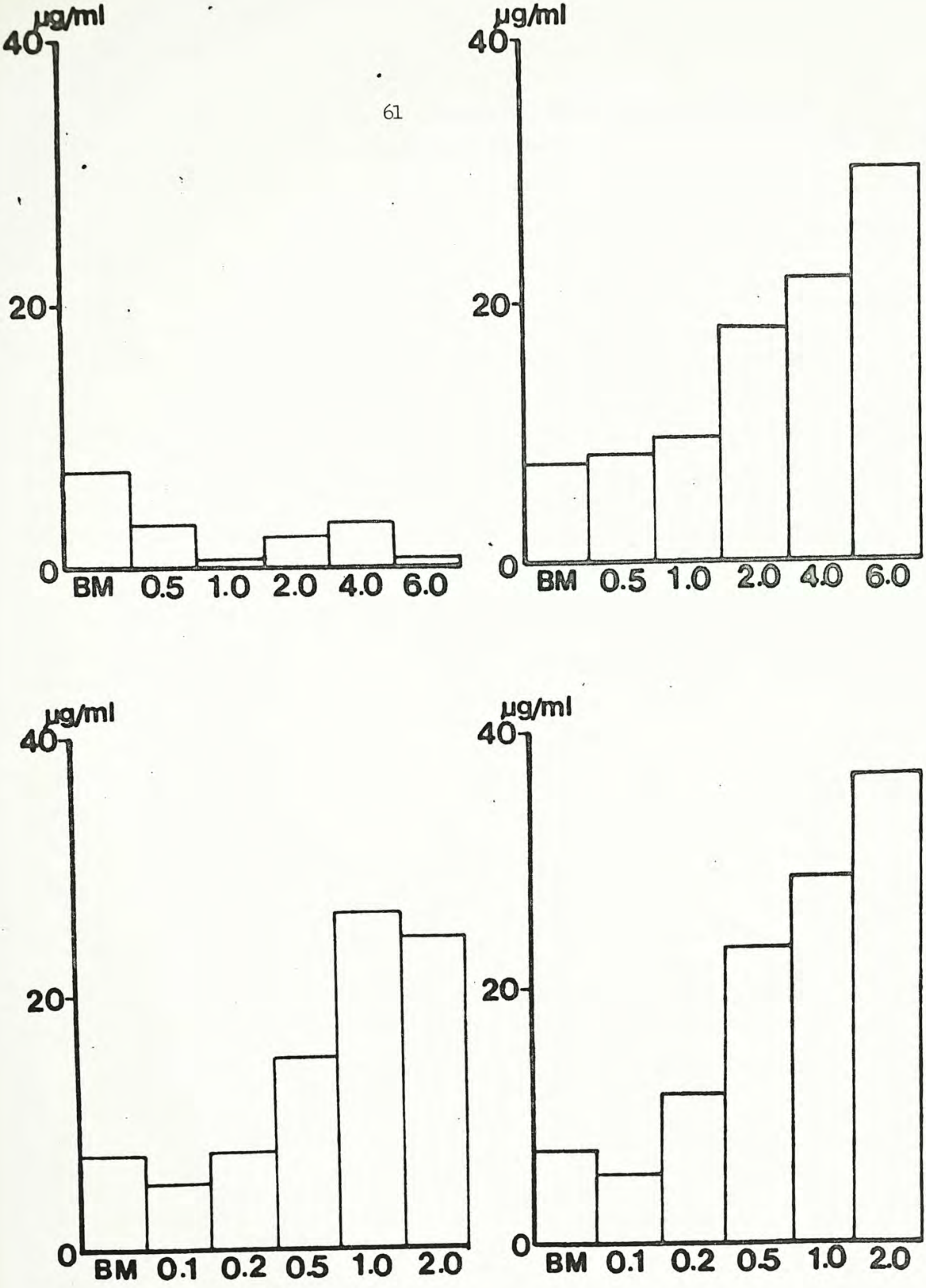


Fig. 3.7 Comparison of chlorophyll content (ug/ml) of harvested Chlorella pyrenoidosa from different culture media

Table 3.2 The metal contents of algal products harvested from extracts of different concentrations of wastes

| | Lead (ppm) | Copper (ppm) | Zinc (ppm) | Manganese (ppm) |
|---|---|---|---|---|
| Activated Sludge | | | | |
| 0.5% | 143.7 | 1796.0 | 3017.2 | 287.4 |
| 1.0% | 943.4 | 3655.7 | 6603.8 | 707.5 |
| 2.0% | 297.6 | 2753.0 | 3869.0 | 446.4 |
| 4.0% | 219.3 | 1699.6 | 4714.9 | 438.6 |
| 6.0% | 196.9 | 1230.3 | 5413.4 | 492.1 |
| Digested Sludge | | | | |
| 0.5% | 107.8 | 1023.7 | 862.1 | 323.3 |
| 1.0% | 74.4 | 1376.5 | 1339.3 | 297.6 |
| 2.0% | 39.7 | 734.1 | 515.9 | 158.7 |
| 4.0% | 76.2 | 590.7 | 1029.0 | 228.7 |
| 6.0% | 87.7 | 471.5 | 701.8 | 175.4 |
| Chicken Manure | | | | |
| 0.1% | 172.4 | 1077.6 | 3017.2 | 258.6 |
| 0.2% | 49.4 | 765.8 | 1235.2 | 98.8 |
| 0.5% | 89.0 | 689.5 | 711.7 | 177.9 |
| 1.0% | 82.2 | 380.3 | 493.4 | 267.3 |
| 2.0% | 46.6 | 454.8 | 559.7 | 326.5 |
| Pig Manure | | | | |
| 0.1% | 80.1 | 1001.6 | 1201.9 | 160.3 |
| 0.2% | 160.3 | 651.0 | 677.1 | 156.3 |
| 0.5% | 66.0 | 412.3 | 428.8 | 98.9 |
| 1.0% | 116.0 | 362.5 | 319.0 | 116.0 |
| 2.0% | 67.5 | 210.9 | 354.3 | 168.7 |
| Bristol Medium | 77.2 | 733.0 | 925.9 | 154.3 |

## 3.4 DISCUSSION

A two-way analysis of variance was made on the six measured parameters (oven-dried weight, chlorophyll content and the contents of the four heavy metals) of the harvested algae.

Only the common concentrations are compared (i.e. 0.5, 1 and 2%). It is found that the harvested algal products are different ($p < 0.05$) only in the type of waste being used but not the concentrations of the wastes. Probably the range of concentrations of the extracts may be too narrow to show any significant difference.

There was no difference ($p > 0.05$) in chlorophyll content, contents of Pb and Mn in the algal products, but significant differences ($p < 0.05$) in oven-dried weight and contents of Cu and Zn were noted in algal products from different types of wastes.

Duncan's multiple range test is further applied to test the difference of oven-dried weight, Zn and Cu contents in the algal products produced by the four wastes (Table 3.3). In general, Table 3.3 shows that the two types of manure and the two types of sludge are similar.

Activated sludge extracts produced algae with the lowest oven-dried weight and the highest concentrations of Cu and Zn concentration. This can be explained by the low levels of essential nutrients such as nitrogen content and extractable phosphate. The extremely high Zn concentration in the

Table 3.3 Results of Duncan's multiple range test on the oven-dried weight, copper and zinc content of algal products harvested from different waste extracts (p=0.05)

| | |
|---|---|
| **Oven-dried weight** | |
| 0.5% | Activated Sludge, Digested Sludge, Chicken Manure $<$ Pig Manure |
| | Activated Sludge $<$ Digested Sludge $<$ Chicken Manure, Pig Manure |
| 1.0% | Activated Sludge, Digested Sludge $<$ Chicken Manure, Pig Manure |
| **Copper content** | |
| 0.5% | Pig Manure, Chicken Manure, Digested Sludge $<$ Activated Sludge |
| | Pig Manure $<$ Chicken Manure $<$ Digested Sludge, Activated Sludge |
| 1.0% | Pig Manure, Chicken Manure $<$ Digested Sludge $<$ Activated Sludge |
| 2.0% | Pig Manure, Chicken Manure, Digested Sludge $<$ Activated Sludge |
| **Zinc content** | |
| 0.5% | Pig Manure, Chicken Manure, Digested Sludge $<$ Activated Sludge |
| 1.0% | Pig Manure, Chicken Manure, Digested Sludge $<$ Activated Sludge |
| 2.0% | Pig Manure, Chicken Manure, Digested Sludge $<$ Activated Sludge |

activated sludge extracts (6.2 ppm) compared with that in other media (below 1 ppm for the extracts of the other three wastes and below 0.1 ppm for Bristol medium) possibly account for the inferior algal growth.

Chlorella is very sensitive to heavy metals. It has been revealed that heavy metals especially Cu, Zn and Mn can greatly inhibit the cell division rate, the photosynthesis, the carbon uptake and respiration. Chlorosis and even cell lysis are common phenomena due to the metal toxicity (Christensen and Scherfig, 1979; DeFillippis and Pallaghy, 1976a, 1976b; Gibson, 1972; Kanazawa and Kanazawa, 1969; McBrien and Hassel, 1967; Nielsen and Nielsen, 1970).

On the other hand, pig manure extracts produced algae with the highest oven-dried weight and the lowest concentrations of Cu and Zn. Digested sludge extracts produced algae similar to those of activated sludge extracts whilst the two types of manure extracts especially pig manure produced excellent algal products with high yields and lower metal contents.

The present study demonstrated that animal manure extracts supported better growth for Chlorella pyrenoidosa than sewage sludge extracts. Harvested algal products from sewage sludge extracts had higher Cu and Zn contents and a lower oven-dried weight than that from animal manure extracts. The low heavy metal content and rich nutrients in the animal manures suggests they are safer for re-utilization. The addition of chicken manure to fish ponds supports growth of lower organisms which serve as food for higher trophic levels. This is a general practice amongst the farmers of Hong Kong.

It has been found that the true protein content (42%) of Chlorella vulgaris harvested from pig manure culture is higher than that of most other plant products. The protein is rich in lysine but deficient in sulphur amino acids (Garrett et al., 1976). Barlow et al. (1975) cultured Chlorella vulgaris in pig manure and produced algal products with a crude protein level around 50%. He then used the algae to feed rats and found that the algal product was a good protein source considering with the protein efficiency ratio and the growth rate of the rats. It compares very favourably with cottonseed meal which is used extensively as a livestock feed. Pig manure has also been used to culture Arthrospira platensis, a spiral blue-green alga subsequently for feeding to rats. Liver, kidney and other organs of the rats were histologically normal (Chung et al., 1978). The above evidences support the possibility of single cell protein production from manure and show a promising future for recycling agricultural wastes.

It has been noted that Euglena gracilis cultivated in the aqueous extracts of activated sludge and digested sludge had significantly high uptake of Fe, Mn and Zn especially Zn when compared with those cultivated in Bristol medium (Wong, 1980). Problems will arise if the algal products are used as animal feed. Certain metals may transfer to higher trophic level as demonstrated by Tam (1979) where algae were cultivated in the aqueous extracts of sludge and in turn used for feeding shrimps and then common carps. Perhaps, cultivation of algae in sewage sludges should be used solely for the purification purpose.

# CHAPTER FOUR

## Animal manures and sewage sludge as supplementary feeds for the growth of the common carp, *Cyprinus carpio*

### 4.1 INTRODUCTION

In Far East, fish pond systems have traditionally been utilized as receptacles for human and animal wastes for production of cheap animal protein (Bardach et al., 1972; Hoffmann, 1934; Korringa, 1976; McGarry and Stainforth, 1978; Sebasrian, 1972; Tang, 1970; Taipiador et al., 1977; Wolfarth and Schroeder, 1979). Polyculture (stocking ponds with different species of fish including silver carp, grass carp, big-head carp, common carp, mud carp, tilapia and mullet) showed the potential advantage for more rational manure utilization (Chislov and Chenokov, 1974; Woynarovich, 1976).

Experimental results showed variations in response of fish upon incorporation of animal manure in the diet. Faster growth rates resulted in studies using chicken manure for goldfish (Lu and Kevern, 1975), tilapia (*Tilapia aurea*) (Burns and Stickney, 1980; Stickney et al., 1977) and channel catfish (*Ictalurus punctatus*) (Fowler and Lock, 1974). Inferior growth rates resulted in studies using chicken manure for common carp (*Cyprinus carpio*) (Kerns and Roelofs, 1977), channel catfish (Lu and Kevern, 1975) and tilapia (Stickney et al., 1977).

Association of pig farms with fish ponds was also found able to increase the daily production of fish. In a polyculture pond of silver carp, common carp and big-head carp, when the number of pigs was increased from 39 to 66 per hectare of pond, the daily fish production increased from 17 to 22 kg per hectare (Buck et al., 1976, 1978). Similar results were also found in studies on the culture of tilapia (Stickney et al., 1977, 1979; Stickney and Hesby, 1978), tilapia hybrids (Lovshin and Da Silva, 1975) and tilapia and Clarias (Nugent, 1978).

In Hong Kong where problems of manure disposal occured (Buck, 1977), 100 pigs or 2,500 ducks kept in pens overhanging the water were considered adequate to fertilize one hectare of carp pond with yields up to 8,000 kg/ha of fish per year under favourable conditions (Bardach et al., 1972).

For domestic wastewater treatment, aquaculture including fish farming is a feasible method which recovers wastes in the form of plant and animal products for a range of uses (Tortell, 1979). Prior to the Second World War, all the rainbow trout sold in England came from Munich wastewater aquaculture system (McGarry, 1977).

Variations in response of fish upon incorporation of sewage sludge in the diet were also noted. Silver carp, big-head carp (Sin and Chin, 1977), common carp (Sin and Chin, 1977; Wong and Kwan, 1981) and rainbow trout (Tacon and Ferns, 1976) grew well while grass carp (Sin and Chin,

1977) and common carp (Kerns and Roelofs, 1977; Wong nad Cheung, 1980; Wong and Kwan, 1981; Yip and Wong, 1977) showed retarded growth. Contradictory results in studies on common carp were due to the proportion of activated sludge incorporated in the diet. In the study conducted by Wong and Kwan (1981), it was noted that only lower proportion of activated sludge (10% and 40%) had weight increase while higher proportion (70% and 85%) produced adverse effect.

Yip and Wong (1977) warned against the use of sewage sludges as pond fertilizer, mainly because of the possibility of heavy metal contamination. This same concern was voiced by Ruttle (1977). Heavy metals present in sewage sludges are in particulate form rather than in solution form. Thus, there is a risk for the fish to ingest them (Sommers, 1977). Organs of common carp grown on activated sludge had incorporated heavy metals but the level of uptake differed among organs (viscera > head and bone > flesh) (Wong and Kwan, 1981). When digested sludge was utilized for the growth of common carp, only very low concentration (0.2%) could be used. Higher concentrations resulted in asphyxiation and finally mortality of fish with histopathological symptoms in various internal organs (Wong et al., 1979). Besides, protein and carbohydrate contents of common carp fed with activated sludge also decrease (Wong and Cheung, 1980).

The aim of the present investigation was two-folded. First, the survival of common carp fed with various concentrations of activated sludge, digested sludge, chicken manure and pig manure was tested. Second, the differences in incorporating these wastes in fish diet, in terms of weight production and quality of fish-flesh, were compared.

## 4.2 MATERIALS AND METHODS

### A Collection and treatment of samples of sewage sludge, animal manures and control diet

Activated sludge was obtained from the sewage treatment plant at The Chinese University of Hong Kong. The manure samples, including chicken manure and pig manure, were collected from farms in the New Territories. They were dried under the sun for one week and grinded by a hand-driven grinder. Both sewage sludge sample and animal manure samples were sieved through a 2 mm sieve before use.

The control diet chosen for this study was a commercial fish food in pellet form for oriental aquarium carps sold under the trade name ' "Echigo" Brand (regular) : New Fish Food'. The constituents of the diet reported by the manufacturer were crude protein minimum 35%, crude fat minimun 4%, crude fibre maximum 4% and crude ash maximum 11%. Pellets were grinded by a pestle and a mortar before use.

### B Culture of fish

Common carps (Cyprinus carpio), measuring around 3 cm bought from local fish farmers, were used in this experiment. Rectangular plastic tanks measuring 60 cm (length) x 40 cm (width) x 20 cm (depth) covered with nylon gauze were used as culture tanks. Compressed air was supplied to each tank throughout the test via submerged 2.5 $cm^3$ airstone.

C Test of mortality against concentration of wastes

Activated sludge, chicken manure and pig manure were added in water in a series of five concentrations (0.1, 0.18, 0.32, 0.56 and 1%) chosen by Probit analysis for testing the mortality of the fish against the concentration of wastes. Eight fish were used in each of the sixteen treatments including a control group which received only the control diet. Dead fish which showed cessation of all visible movement were removed during daily check. Number of fishes removed was recorded for a period of nine days.

D Rearing of fish using wastes as a feed supplement

Wastes were added to the control fish diet as a feed supplement in a series of proportions (20, 40, 60, 80 and 100%). Five carps were used in each of the sixteen treatments including a control group fed with 100% control fish diet. The carps were fed on alternate days when water was changed with amounts equal to 3% of body weight. Body weights of fish in each treatment were recorded weekly for a period of four weeks. At the end of the culture period, all the fish were collected, descaled and the flesh was obtained for analysis.

E Analysis of fish-flesh

Physical analysis including fresh weight, oven-dried weight (at 105°C for 24 hours) and ash weight (at 500°C for 3 hours) were conducted. Nutritive analysis including protein content (Lowry et al., 1951), soluble-carbohydrate content (Dreywood, 1946) and extractable phosphate content (dry ashing method (Allen et al., 1974) followed by molybdenum blue method (Watanable and Olsen, 1962)) were carried out. Lipid content was determined by charring method (Kritchevsky et al., 1973). Ash-dried flesh was further processed by dry ashing method (Allen et al., 1974) before total heavy metal contents were analysed using an atomic absorption spectrophotometer.

## 4.3 RESULTS

A Mortality study

Figures 4.1, 4.2 and 4.3 showed the mortality of common carp (Cyprinus carpio) in different concentrations of activated sludge, chicken manure and pig manure. $LC_{50}$ (the lethal concentration which kills 50% of the test animals) values at 48, 96, 144 and 192 hour intervals with corresponding 95% confidence level upper and lower limit were obtained by Spearman-Kärber method (Finney, 1971) (Table 4.1).

The $LC_{50}$ values in the treatment with pig manure were 0.92% (48 hr.), 0.55% (96 hr.), 0.28% (144 hr.) and 0.18% (192 hr.), all differed from each other significantly ($p < 0.05$) (Table 4.1). In the treatment with chicken manure, the values were 0.47% (48 hr.), 0.29% (96 hr.), 0.17% (144

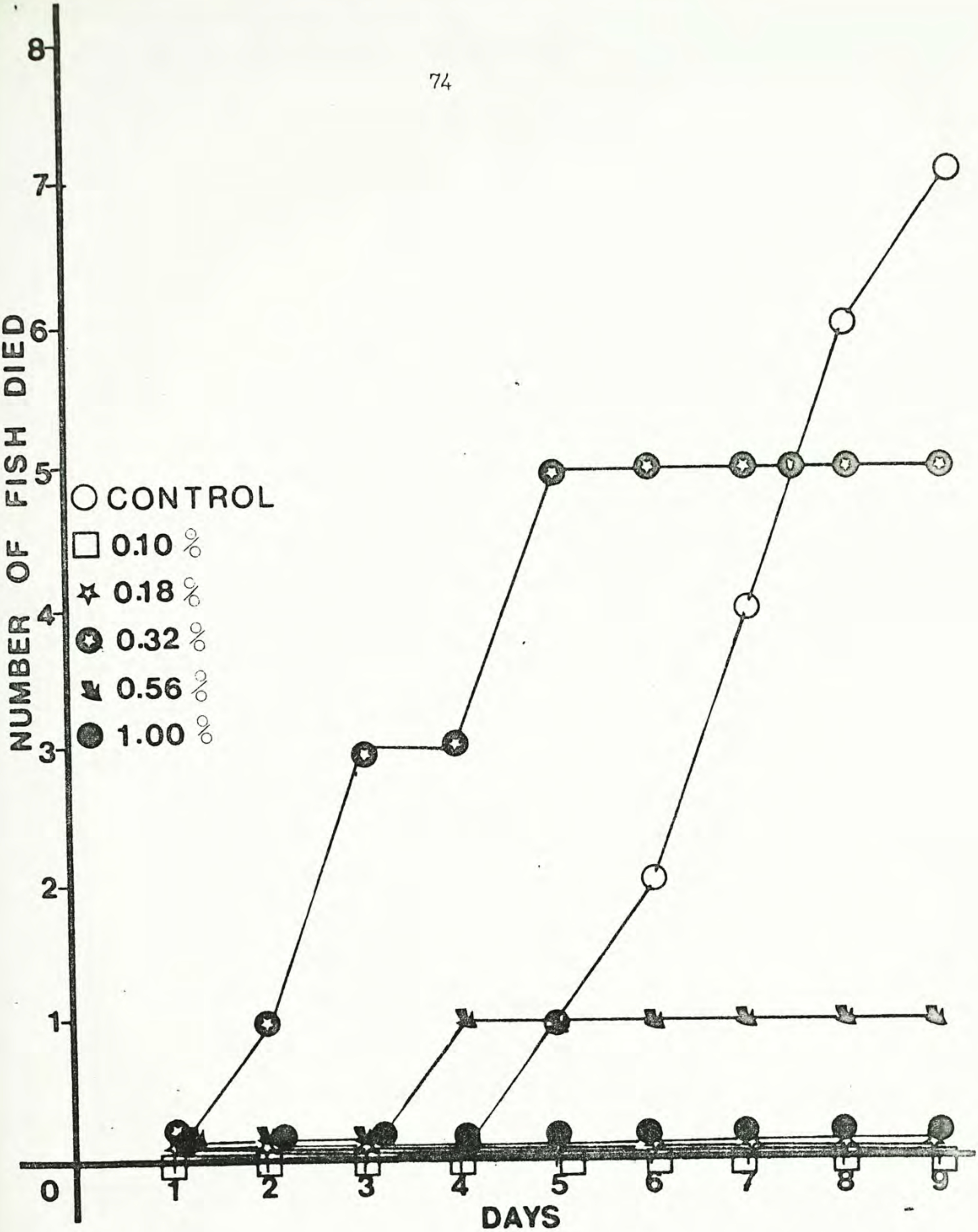


Fig. 4.1 Mortality of common carp in different concentrations of activated sludge

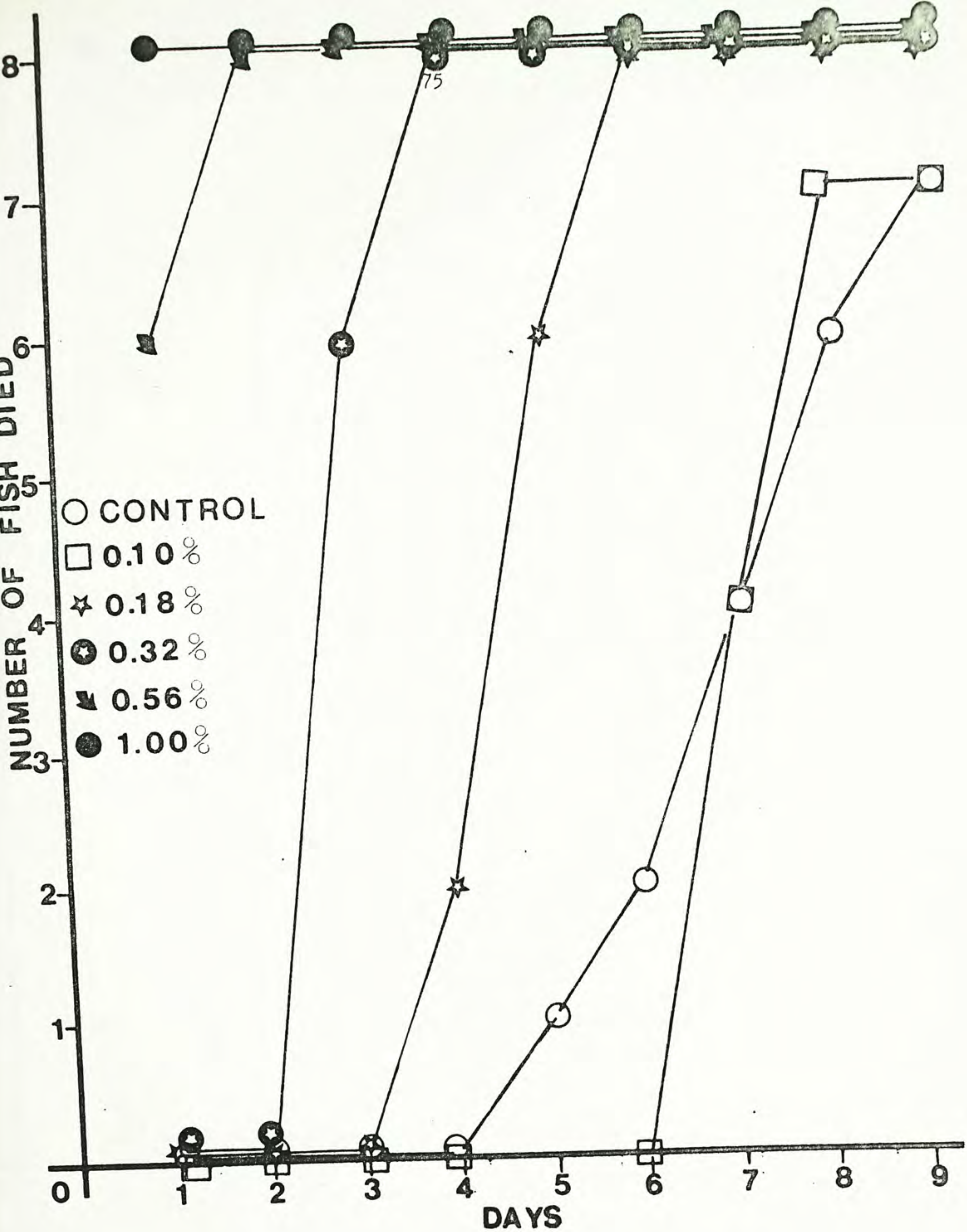


Fig. 4.2 Mortality of common carp in different concentrations of chicken manure

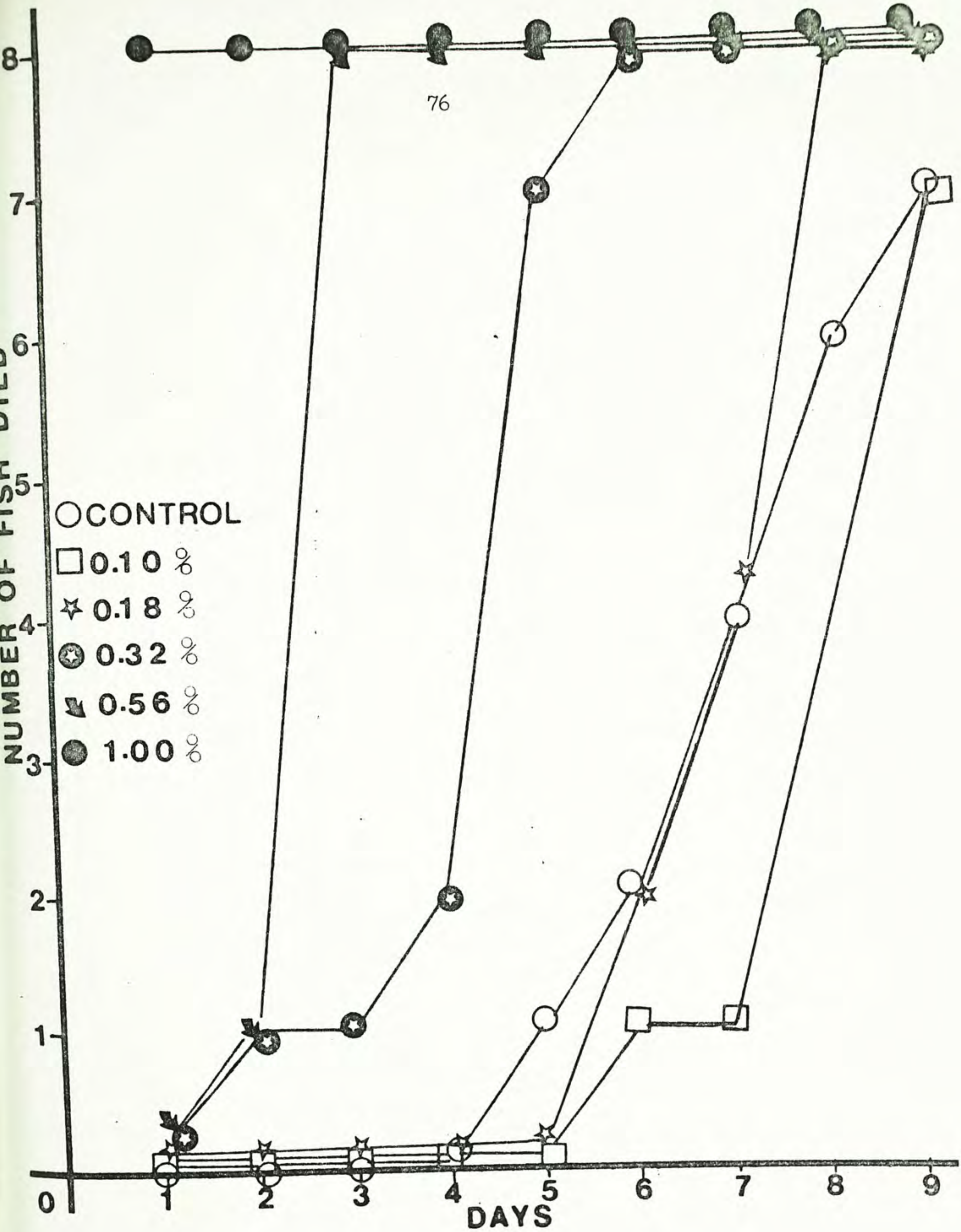


Fig. 4.3 Mortality of common carp in different concentrations of pig manure

Table 4.1 $LC_{50}$ values, of activated sludge, chicken manure and pig manure, with their 95% upper and lower limits, calculated for periods of 48, 96, 144 and 192 hours using Spearman-Kärber method (Finney, 1971).

| | 48hr. | 96hr. | 144hr. | 192hr. |
|---|---|---|---|---|
| Activated Sludge | | | | |
| $LC_{50}$ | nev | nev | nev | nev |
| 95% limit | | | | |
| upper | nev | nev | nev | nev |
| lower | nev | nev | nev | nev |
| Chicken Manure | | | | |
| $LC_{50}$ | 0.4700 | 0.2900 | 0.1650 | 0.1370 |
| 95% limit | | | | |
| upper | nev | 0.3100 | 0.1710 | 0.1450 |
| lower | nev | 0.2800 | 0.1590 | 0.1290 |
| Pig Manure | | | | |
| $LC_{50}$ | 0.9175 | 0.5475 | 0.2000 | 0.1750 |
| 95% limit | | | | |
| upper | 0.9556 | 0.5690 | 0.2980 | 0.1880 |
| lower | 0.8790 | 0.4958 | 0.2620 | 0.1650 |

nev : no effective value

hr.) and 0.14% (192 hr.), all differed from each other by 0.05 significance level except the value of 48 hr. (Table 4.1). No $LC_{50}$ value could be obtained in activated sludge treatment since most of the fish survived after a duration of 192 hr.. In general, $LC_{50}$ values became smaller as the duration of treatment became longer.

B Body weight changes of fish during growth period

Figures 4.4, 4.5 and 4.6 compared the changes of body weight of common carp fed with various waste-added diet compared with the control group. Experimental results showed that all the three wastes were not satisfactory for being used as feed supplements for the common carp. Body weight decreased in all the fifteen treatments except the pig manure 20% group (+ 2.1%). Control group had a remarkably increase in body weight (+ 15.9%) which further indicated the inferior growth condition when waste was incorporated in the fish diet. Furthermore, the higher proportion of waste in the diet, the lower final weights were obtained. For example, in activated sludge treatments 20, 40, 60, 80 and 100% incorporation yielded 97.5, 86.8, 89.8, 81.2 and 67.1% final body weights respectively (Fig. 4.1). Similar trends were also found in animal manure treatments (Figs. 4.2 and 4.3).

C Characteristics of flesh of fish finally harvested

Physical characteristics (Table 4.2), nutritive levels (Table 4.3) and heavy metals contents (Table 4.4) of flesh

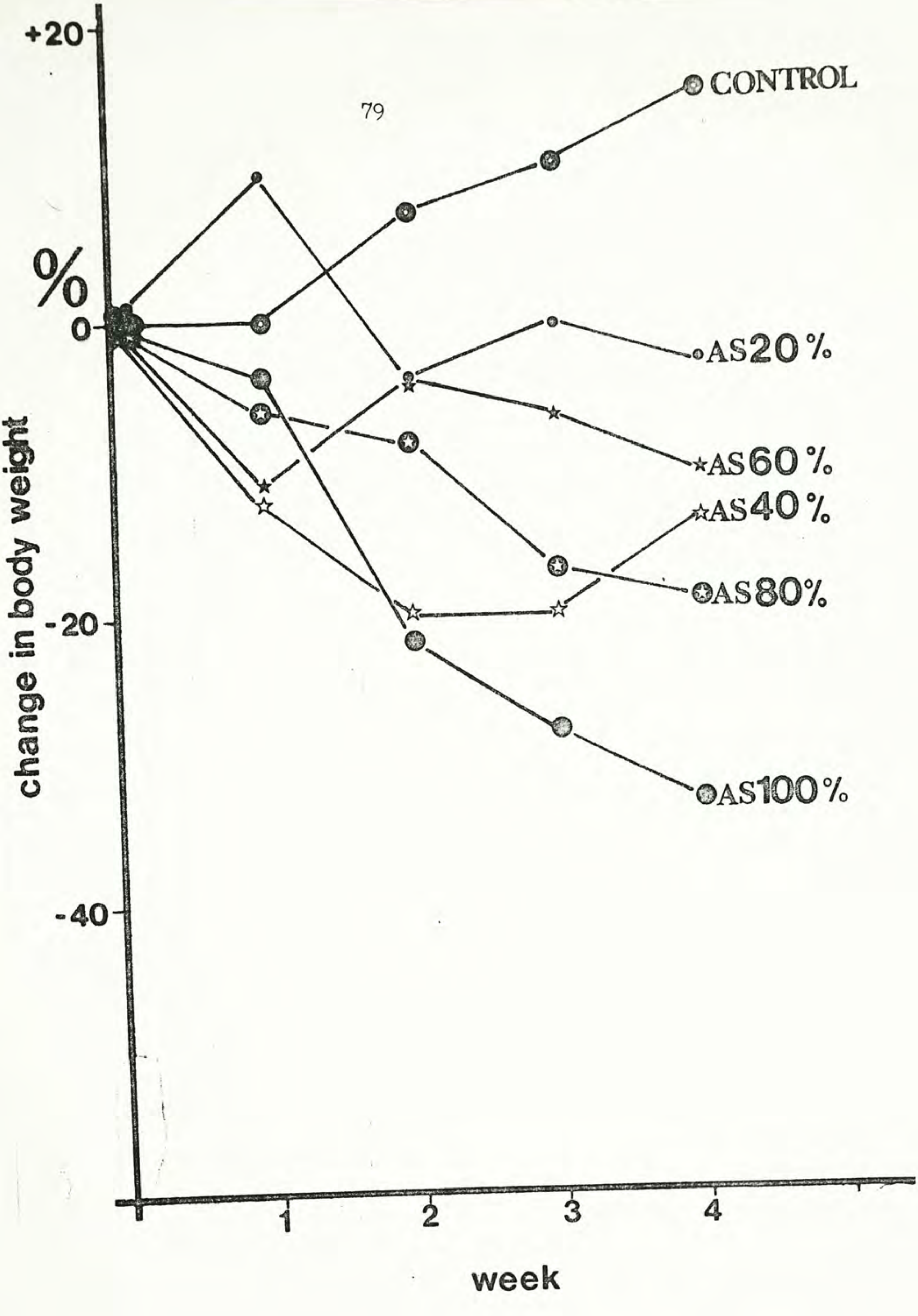


Fig. 4.4 Changes of body weight of fishes fed with various portions of activated sludge diet within a period of four week

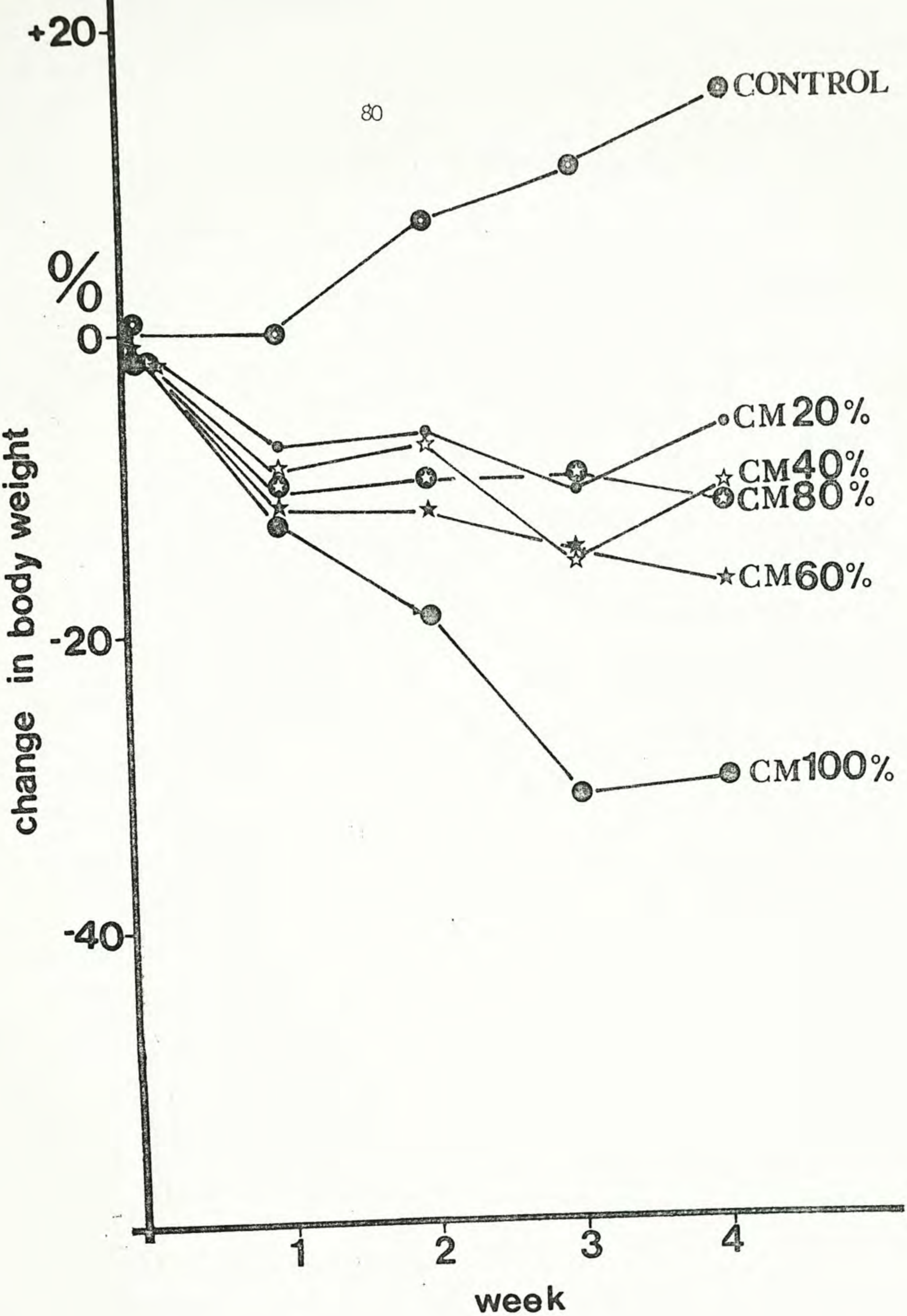


Fig. 4.5 Changes of body weight of fishes fed with various portions of chicken manure diet within a period of four week

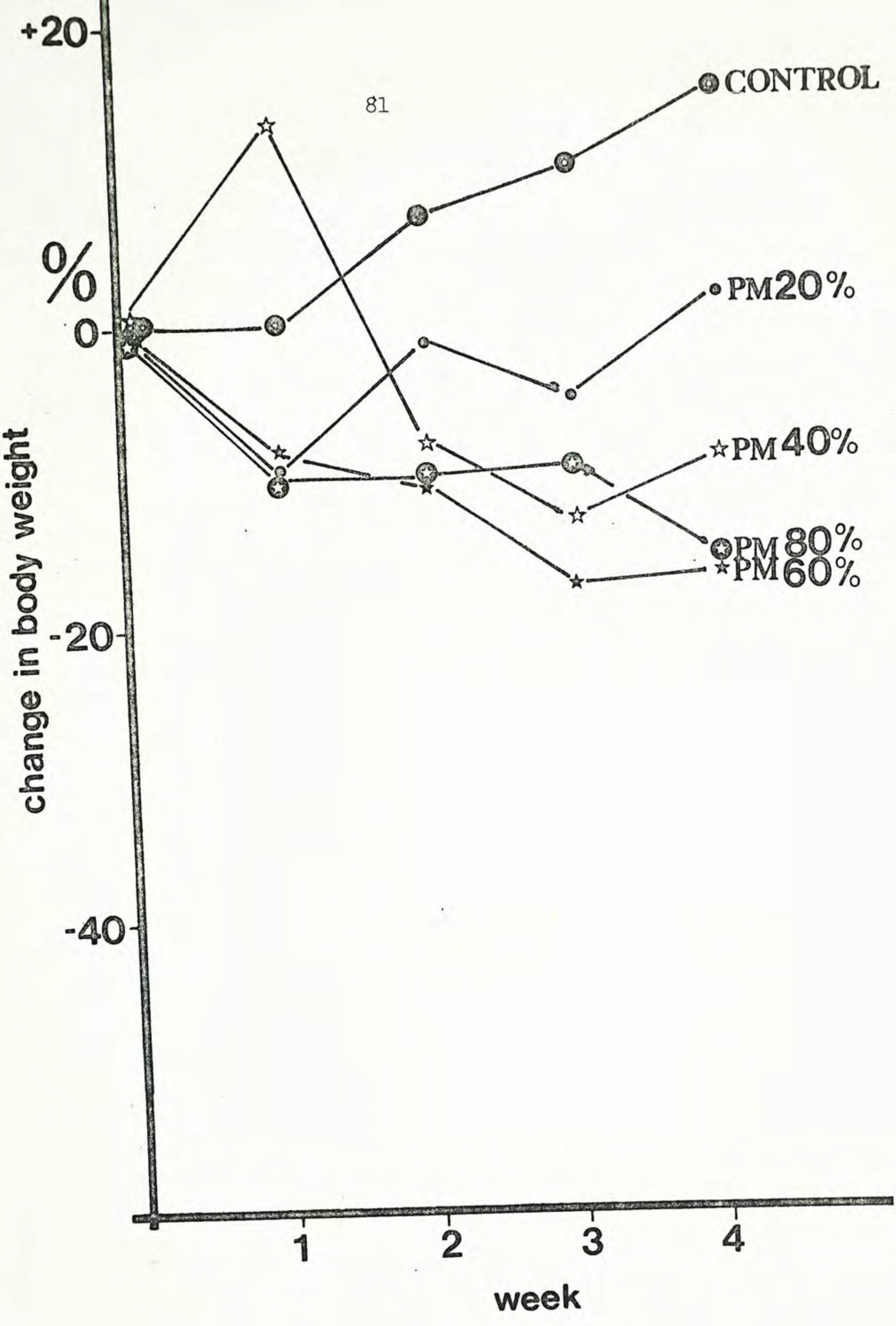


Fig. 4.6 Changes of body weight of fishes fed with various portions of pig manure diet within a period of four week

Table 4.2 Physical characteristics of the flesh of fish finally harvested from various treatments, values were in percentage.

| Treatment | Air-dried weight / Fresh weight (%) | Oven-dried weight / Fresh weight (%) | Ash weight / Oven-dried weight (%) |
|---|---|---|---|
| Control | 26.97 | 21.91 | 9.11 |
| Activated Sludge | | | |
| 20% | 26.71 | 21.63 | 12.29 |
| 40% | 24.74 | 19.90 | 17.71 |
| 60% | 25.75 | 19.30 | 11.45 |
| 80% | 23.71 | 19.19 | 14.41 |
| 100% | 23.38 | 19.10 | 12.52 |
| Chicken Manure | | | |
| 20% | 26.56 | 21.09 | 10.05 |
| 40% | 27.53 | 22.43 | 10.20 |
| 60% | 25.46 | 20.71 | 14.74 |
| 80% | 25.99 | 20.95 | 10.76 |
| 100% | 19.98 | 16.11 | 21.06 |
| Pig Manure | | | |
| 20% | 27.07 | 21.85 | 10.03 |
| 40% | 25.87 | 21.07 | 12.70 |
| 60% | 23.29 | 21.07 | 13.99 |
| 80% | 21.95 | 17.76 | 15.72 |

Table 4.3 Nutritive levels (in % and ppm of oven-dried weight) of the flesh of fish finally harvested from various treatments.

| Treatment | Protein % | Carbohydrate % | Lipid % | Phosphate ppm |
|---|---|---|---|---|
| Control | 18.20 | 5.63 | 10.29 | 648.33 |
| Activated Sludge | | | | |
| 20% | 24.18 | 6.42 | 9.28 | 692.93 |
| 40% | 23.42 | 5.95 | 7.78 | 775.83 |
| 60% | 29.07 | 3.03 | 6.07 | 1021.13 |
| 80% | 18.54 | 1.67 | 7.22 | 876.97 |
| 100% | 24.68 | 0.48 | 6.60 | 751.15 |
| Chicken Manure | | | | |
| 20% | 18.73 | 4.80 | 9.39 | 627.22 |
| 40% | 15.02 | 2.71 | 7.78 | 671.20 |
| 60% | 16.53 | 3.91 | 8.28 | 698.21 |
| 80% | 20.65 | 1.54 | 7.05 | 1145.87 |
| 100% | 23.26 | 0.40 | 8.30 | 1039.54 |
| Pig Manure | | | | |
| 20% | 22.97 | 4.82 | 6.32 | 746.13 |
| 40% | 22.60 | 2.55 | 7.72 | 754.49 |
| 60% | 18.19 | 3.53 | 9.65 | 662.73 |
| 80% | 23.37 | 0.91 | 9.31 | 1231.08 |

Table 4.4 Heavy metal contents (ppm) of the flesh of fish finally harvested from various treatments.

| Treatment | Lead (ppm) | Copper (ppm) | Zinc (ppm) | Manganese (ppm) | Cadmium (ppm) |
|---|---|---|---|---|---|
| Control | 1.57 | 5.08 | 86.86 | 7.86 | n.d. |
| Activated Sludge | | | | | |
| 20% | 3.36 | 13.89 | 106.70 | 21.73 | 1.10 |
| 40% | 2.27 | 15.15 | 155.34 | 21.46 | 0.37 |
| 60% | 3.65 | 7.18 | 98.41 | 14.94 | 0.41 |
| 80% | 3.18 | 6.61 | 143.67 | 16.10 | 0.74 |
| 100% | 2.45 | 5.51 | 109.28 | 14.86 | 0.56 |
| Chicken Manure | | | | | |
| 20% | 9.37 | 3.58 | 70.12 | 3.18 | 0.17 |
| 40% | 1.27 | 3.56 | 84.26 | n.d. | 0.20 |
| 60% | 3.36 | 12.29 | 126.88 | 11.21 | n.d. |
| 80% | 0.66 | 10.98 | 69.61 | 3.19 | n.d. |
| 100% | 2.27 | 8.43 | 136.48 | 20.49 | 0.53 |
| Pig Manure | | | | | |
| 20% | 2.21 | 7.39 | 87.24 | 13.85 | n.d. |
| 40% | n.d. | 4.59 | 200.92 | 23.91 | 1.02 |
| 60% | n.d. | 12.75 | 170.40 | 21.18 | 0.95 |
| 80% | 0.76 | 8.85 | 156.33 | 30.36 | 2.35 |

n.d. denoted non detectable

of fish harvested from various treatment further confirmed that commercial fish feed was more superior than any of the wastes being used as supplementary feeds. Higher percentages of air-dired weight / fresh weight and oven-dried weight / fresh weight, higher contents of carbohydrate and lipid; lower percentage of ash weight / oven-dried weight and lower levels of heavy metal contents were found in the control treatment. The only inferiority was the content of protein. All the three wastes showed inferior growth upon incorporation as supplementary feeds.

The results of the pig manure 100% treatment were doubtful and rejected because of the extraordinary data (e.g. 42.63% protein, 41.18% lipid). Such results were probably due to the small sample weights (0.15 gm compared with 0.75 gm used for other treatments) of fish flesh used in the analysis.

The differences in characteristics of fish flesh among the three wastes being used as supplementary feeds were compared. Concerning with physical characteristics of the flesh, manure-fed fish had higher percentages of air-dried weight / fresh weight, oven-dried weight / fresh weight and ash weight / oven-dried weight (Table 4.2). Similar trends were also found in lipid and phosphate levels. However, when protein and carbohydrate levels were compared, activated sludge-fed fish was found to have higher levels (Table 4.3).

The patterns of difference in heavy metals among the wastes were somehow dependent on different metals. Activated sludge-fed fish had higher contents of Pb, Cu and Cd but lower Zn and Mn when compared with those fed by pig manure. However, the lowest level of heavy metals was obtained in chicken manure-fed fish (Table 4.4). Generally speaking, all the waste resulted in the accumulation of heavy metals in the fish-flesh. For example 1.4 - 2.3 fold of Pb, 2.4 - 3.0 fold of Cu, 1.6 - 2.3 fold of Zn and 2.6 - 4.0 fold of Mn were noted in the waste-treated fish when compared with the control. Besides, all the three wastes showed an increase in Cd content while it was non-detectable in the control group.

## 4.4 DISCUSSION

A comparison of the data on mortality indicated that the decending order of toxicity of the wastes were : pig manure, chicken manure and activated sludge. Such observations were expected since activated sludge was the product of aerobic digestion from the sewage treatment plant, while the animal manure had not passed through any decomposition process. Furthermore, the animal manure had higher contents in total nitrogen, organic carbon and extractable phosphate than activated sludge (Section 2.3 of this thesis).

Fish reared in the artificial high-grade diet alone had higher carbohydrate and protein levels, but lower levels of heavy metals than that fed with sewage sludges and animal manure thus produced the highest yield. The present study was coincidence with others (Kerns and Roelofs, 1977; Lu and Kevern, 1975; Moav et al., 1977; Shilon and Viola, 1973;

Stickney et al., 1977; Summerfelt and Yin, 1974; Tacon and Ferns, 1976; Woynarovich, 1976).

On the other hand, the present study which failed to demonstrate the positive effect of incorporating wastes in the fish diet could be explained in two aspects.

First aspect concerned with the inadequate processing of the wastes. In early European practice, heaping or spreading the manure over the pond bottom was carried out before filling the pond with water. This resulted in depletion of oxygen, production of hydrogen sulphide and methane and buildup of ammonia in the vicinity of the manure (Woynarovich, 1956a, 1956b,1957, 1976). In the present study, the animal manures used did not undergo any decomposition treatment. When animal manures were applied to the aerated water, the enriched nutrients in the wastes might undergo decomposition and produce ammonia and ethylene. Changes in water quality also included pH and turbidity which all excerted harmful effects on fish.

Second aspect concerned with the modes of action of the wastes. Waste application facilitates growth of aquatic organisms in one out of the three following ways :

a <u>Direct consumption by the fish</u>

If this mode of action was the only way for the fish to utilize the wastes, inferior growth rate should result. All the wastes had lower levels of carbohydrate and protein when compared with high-grade fish feed. Besides, toxic ingredients in the wastes, e.g. heavy metals, even though in sublethal amount, could affect body metabolism and resulted in retarded growth.

b As minerals for photosynthetic production of phytoplankton

In the presence of mineral fertilizers from the wastes, phytoplankton utilzes inorganic ingredients as nutrient pool and solar energy as energy source for primary productivity in biomass (Anon, 1977; Hepher, 1962; Tamiya, 1957). Zooplankton further transferred this biomass to fish fry through predatary food chain.

c As a substance supplying organic and mineral matter to the heterotrophic micro-organisms

Microbial community, bacteria and protozoa, flourishes on organic matter added to the pond, using the organic and mineral fractions as sources of energy and nutrients. Fish harvest these micro-organisms in the form of microbial slimes on detritus or as naturally occuring flocs in the water column (Kuznetsov, 1977; Odum, 1974; Spataru, 1976; Summerfelt et al., 1970).

Grass carp larvae and fry were fed almost exclusively on animal food in pond conditions (Ciborowska, 1972). Even when grass carp reached 36 - 42 mm, chironomid larvae and zooplankton dominated their food intake (Opuszynski, 1969). Common carp fry was noted to feed on natural zooplankton, e.g. rotifers and daphnia (Chen, 1976). However, in this study, the use of plastic tanks and the duration of time did not allow autotrophic or heterotrophic food web to carry out. Therefore it was reasonable to have such results.

Nevertheless, the present study demonstrated the acute effect of the three wastes in a laboratory scale. In natural conditions, nutrients in applied wastes were utilized and transformed into biomass of micro-organisms before being utilized by the fish which acts as the secondary consumer of the food chain. In laboratory study, direct consumption could be the only mode of intake of the wastes into the fish.

Moreover, in small enclosures, toxic ingredients of the wastes, e.g. heavy metals, would affect directly on the fish. In the study conducted by Wong et al. (1979) on the same species using a higher portion of digested sludge (0.2 - 1.2% w/v), heavy metals of the sewage sludge seemed to react with the constituents of the mucus secreted by the gills of the fish. Such coagulation blocked the gill filaments, affected the circulatory systems. Incorporation of heavy metal into kidney and liver of the fish was also found (Wong et al, 1979, Wong and Kwan, 1981). This led to the impairment of organs and disturbing the normal body metabolism.

If wastes were used as feed supplements, the characteristics and the necessary pretreatment of the wastes and the feeding habit of the animal tested should be taken care of.

In Part Two of this thesis, two food chains studies were constructed in order to test the subsequent effects of these wastes on higher trophic levels in food chain.

# CHAPTER FIVE

## Utilization of animal manures and sewage sludges for the growth of Flowering Chinese Cabbage, *Brassica parachinensis*

### 5.1 INTRODUCTION

In research on utilization of sewage sludges on agricultural land, plant species varied widely in response due to their different sensitivity to available heavy metals. Corn (*Zea mays*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) resulted in increased yield while lower yield of beans resulted (Binham, 1976; Giordano et al., 1975; Hinesly and Sosewitz, 1969; Hinesly et al., 1974; Keefer et al., 1979; King, 1974; Kirkham, 1974, Linnman et al., 1973; Lunt, 1969; Lutrick, 1973; Milne and Graveland, 1972). Repeated application of sewage sludges was found to cause damage to the crops through buildup of heavy metals (Patterson, 1971). Cd, Mn and Zn increased in soil and plant tissues of corn, soybean (*Glycine max*) and Flowering Chinese Cabbage (*Brassica parachinensis*) in sewage sludge application (Jones et al., 1973, 1975; Wong and Yip, 1978). Morphologically, apical chlorosis appeared on tomato (*Lycopersicon esculentum*) plants grown on sludge-amended soil (Touchton and Boswell, 1975).

Land spreading of animal manure on agricultural land had been a traditional practice. However, besides its high nutrient content, animal manure also has a high salt content, including large amounts of sodium and bicarbonate. Armstrong (1972) reported that undiluted and diluted piggery effluents contained about 28,000 ppm and 10,000 ppm of total solids respectively. Na, K, Ca, Mg and P were found increased in soil received piggery effluent treatment (Jeffery and Uren, 1979; Kornegay et al., 1976). Furthermore, artificial supplements containing heavy metals are incorporated in pig diet to facilitate maximum growth, e.g. supplementation of Cu in pig diet had shown to promote weight gain and increase food conversion rates of fattening pigs (Barber et al., 1955). Zn is also added to pig diet in order to eliminate any toxicity associated with high copper concentration (Hanraham and O'Grady, 1968). Only a small percentage (5 - 10%) of dietary Cu and Zn is absorbed by the pig, the rest is eliminated in pig manure. Application of piggery effluent to pasture therefore resulted in accumulation of copper in surface soil layer and further in plant tissue (Batey et al., 1972; Jeffery and Uren, 1979; McAllister, 1977; Woodside, 1973).

The aim of this experiment is to compare the suitability of activated sludge, digested sludge, chicken manure and pig manure for the use as soil additive for vegetable growth. The vegetable chosen for the test is Chinese Flowering Cabbage, *Brassica parachinensis*, which is an important vegetable in Hong Kong.

## 5.2 MATERIALS AND METHODS

### A Collection and treatment of samples of sewage sludges and animal manures

The sewage sludges samples were obtained from two sites : activated sludge from the Sewage Treatment Plant at The Chinese University of Hong Kong and digested sludge from Shek Wu Hui Sewage Treatment Plant. The animal manure samples including chicken manure and pig mnure were collected from farms in the New Territories of Hong Kong. Both samples were dried under sun for one week and grinded by a hand-driven grinder. They were then sieved through a 2 mm sieve before use.

### B Cultivation of vegetables

Plastic pots filled with soil were used for the growth of the vegetable. Application of samples of sewage sludges and animal manures to the pots was based on a weight per unit area ratio. Rates of applocation chosen were 0.1125, 0.4500 and 2.2500 kg/$m^2$ (i.e. 0.5, 2 and 10 ton/acre) (Table 5.1). Pots after applied with the wastes were irrigated daily with water for one week before seeds of *Brassica parachinensis* were sown.

About fifty deeds were sown to each pot and three weeks later only two of the strongest seedlings were chosen for further growth. The vegetables were harvested five weeks later.

Table 5.1 Explanation of abbreviations of various treatment in this study.

| | |
|---|---|
| CONT | Control group, without any waste added |
| | Activated Sludge applied |
| AS 5 | 0.1125 kilogram/square meter (0.5 ton/acre) |
| AS 20 | 0.4500 kilogram/square meter (2.0 ton/acre) |
| AS100 | 2.2500 kilogram/square meter (10.0 ton/acre) |
| | Digested Sludge applied |
| DS 5 | 0.1125 kilogram/square meter (0.5 ton/acre) |
| DS 20 | 0.4500 kilogram/square meter (2.0 ton/acre) |
| DS100 | 2.2500 kilogram/square meter (10.0 ton/acre) |
| | Chicken Manure applied |
| CM 5 | 0.1125 kilogram/square meter (0.5 ton/acre) |
| CM 20 | 0.4500 kilogram/square meter (2.0 ton/acre) |
| CM100 | 2.2500 kilogram/square meter (10.0 ton/acre) |
| | Pig Manure applied |
| PM 5 | 0.1125 kilogram/square meter (0.5 ton/acre) |
| PM 20 | 0.4500 kilogram/square meter (2.0 ton/acre) |
| PM100 | 2.2500 kilogram/square meter (10.0 ton/acre) |

Since there were four samples of wastes and each had three rates of application, therefore there were totally thirteen different treatments and twenty-six pots of growth in the green house trial.

C Analysis of soil

Before the sowing of seeds, soil samples were taken at 2 cm below surface for analysis. They were air-dried and passed through a 2 mm sieve before the following analysis were carried out : pH (pH meter, 10 gm soil : 25 ml distilled water), conductivity (conductivity meter, 10 gm soil : 25 ml distilled water), organic carbon (Walkey and Black, 1934) and extractable phosphate (extractant : 2.5% acetic acid, molybdenum blue method, Watanabe and Olsen, 1962). The heavy metals (mixed acid digestion, $HClO_4$ : $HNO_3$ : $H_2SO_4$ = 2 : 10 : 1) and exchangeable heavy metals (extractant : 1 M ammonium acetate solution at pH 7) were also measured using an atomic absorption spectrophotometer following the methods described by Allen et al. (1974).

D Analysis of vegetables

The gross morphological description including root length, shoot length, total fresh weight, number of leaves of each plant, and percentage of plants flowered were recorded. Further analysis included total dry matter production (at 105°C for 48 hours), fresh and oven-dried weights of the plants. The heavy metal contents of the various tissues of the plants were determined by mixed acid digestion ($HClO_4$ : $HNO_3$ : $H_2SO_4$ = 2 : 10 : 1) and measured by an atomic absorption spectrophotometer following the methods described by Allen et al. (1974).

E Anatomical study on leaf sections

Leaf sections were cut during harvest and processed in paraffin section for anatomical study. Slides were counter-stained with Delafield's hematoxylin and eosin. Photographs were taken under a compound light microscope.

## 5.3 RESULTS

A Effect of addition of wastes on edaphic properties (Table 5.2)

a Addition of sewage sludges lowered the pH of the soil while addition of animal manures showed an opposite effect. Soil pH values were lower (around 5.8) in sewage sludge-added soils and higher (around 7.3) in animal manure-added soils.

b Manured soils showed higher conductivity values (441 µS in PM 100 soil, 921 µS in CM 100 soil). On the other hand, sludge-amended soils showed lower conductivity values (147 µS in AS 100 soil and 214 µS in DS 100 soil). In general, chicken manure-amended soils had higher conductivity than that of pig manure-amended soils, while digested sludge-amended soils had higher conductivity than that of activated sludge-amended soils.

Table 5.2 Properties of soil treated with various concentrations of wastes prior the cultivation of Brassica parachinensis.

| | pH (1:2.5) | Conductivity (1:2.5) (µS) | Organic Carbon (%) | Extractable Phosphate (ppm) |
|---|---|---|---|---|
| CONT | 6.35 | 122 | 3.65 | 20 |
| AS 5 | 6.32 | 102 | 4.26 | 11 |
| AS 20 | 6.12 | 126 | 5.15 | 34 |
| AS100 | 5.80 | 147 | 7.77 | 37 |
| DS 5 | 6.27 | 116 | 4.92 | 69 |
| DS 20 | 6.10 | 147 | 6.08 | 105 |
| DS100 | 5.82 | 214 | 5.98 | 429 |
| CM 5 | 6.55 | 173 | 5.06 | 78 |
| CM 20 | 6.69 | 308 | 5.69 | 420 |
| CM100 | 7.32 | 921 | 6.12 | 742 |
| PM 5 | 6.81 | 111 | 5.47 | 395 |
| PM 20 | 6.88 | 192 | 7.92 | 1810 |
| PM100 | 7.33 | 441 | 7.46 | 1729 |

c The organic carbon content of all the treated soil had no significant difference ($p > 0.05$). However, all of the treated soils showed significantly ($p < 0.05$) higher organic carbon content than the untreated soils (control).

d Similar results were also noted in extractable phosphate content. Manure-amended soils had higher level (1,729 ppm in PM 100 soil, 742 ppm in CM 100 soil) of extractable phosphate than that of sludge-amended soil (429 ppm in DS 100 soil and 37 ppm in AS 100 soil). However, concerning with the difference among the animal manures, pig manure-amended soils had higher level than that of chicken manure-amended soil, a reverse order as compared with that shown in conductivity measurements.

B Heavy metal content of soils treated with various concentrations of wastes

Levels of the total and exchangeable contents of heavy metals (Pb, Cu, Zn and Mn) in soils treated with various concentrations of wastes are presented in Tables 5.3 and 5.4 respectively. According to the results of two-way analysis of variance, there were no significant difference ($p > 0.05$) among different wastes as well as different concentrations of wastes applied on the contents of total heavy metals.

Table 5.3 Total heavy metal content (ppm) of soils treated with various concentrations of wastes prior the cultivation of <u>Brassica parachinensis</u>, each value was a mean ± standard deviation.

| | Pb | Cu | Zn | Mn | Sum |
|---|---|---|---|---|---|
| CONT | 128.00±17.56 | 22.33±15.84 | 68.00± 23.12 | 116.33± 27.18 | 334.66 |
| AS 5 | 92.13±17.13 | 45.00± 5.00 | 82.00± 17.68 | 191.25± 25.83 | 410.38 |
| AS 20 | 114.50±17.97 | 32.50±22.78 | 51.00± 25.97 | 99.50± 37.92 | 297.50 |
| AS100 | 139.25±21.70 | 50.00±18.71 | 67.50± 29.28 | 188.25± 96.21 | 445.00 |
| DS 5 | 121.75±40.89 | 14.25± 9.81 | 89.00± 4.95 | 462.50±234.10 | 687.50 |
| DS 20 | 118.00±39.04 | 25.00±15.00 | 81.00± 28.77 | 273.75±127.69 | 497.75 |
| DS100 | 88.00±24.06 | 86.75±47.92 | 204.50±138.50 | 281.25± 79.95 | 660.50 |
| CM 5 | 65.00±19.29 | 25.00± 8.66 | 8.33± 5.79 | 71.50± 10.71 | 169.83 |
| CM 20 | 121.25±25.81 | 18.50±11.50 | 42.75± 29.01 | 263.75±143.93 | 446.25 |
| CM100 | 58.25±19.33 | 37.50± 8.29 | 55.50± 25.60 | 211.25± 66.18 | 362.50 |
| PM 5 | 156.50±20.51 | 52.50±10.90 | 70.25± 13.86 | 197.50± 2.50 | 476.75 |
| PM 20 | 143.00±17.56 | 65.00± 8.66 | 55.75± 10.78 | 132.00± 17.87 | 395.75 |
| PM100 | 161.50±38.45 | 75.00±11.18 | 58.75± 5.89 | 183.75± 54.13 | 479.00 |

Table 5.4 Exchangeable heavy metal content (ppm) of soils treated with various concentrations of wastes prior the cultivation of Brassica parachinensis, each value was a mean ± standard deviation.

| | Pb | Cu | Zn | Mn | Sum |
|---|---|---|---|---|---|
| CONT | 51.88±4.43 | 9.58±3.93 | 14.21±0.87 | 63.54± 2.83 | 139.21 |
| AS 5 | 51.75±8.02 | 12.92±2.24 | 21.75±0.90 | 83.54± 2.44 | 169.96 |
| AS 20 | 54.88±9.34 | 20.00±3.23 | 30.29±1.75 | 82.64± 1.84 | 187.81 |
| AS100 | 44.85±7.64 | 24.25±2.87 | 48.50±3.28 | 89.17± 6.11 | 206.50 |
| DS 5 | 44.92±6.38 | 11.67±2.76 | 15.83±2.00 | 71.04±11.35 | 143.46 |
| DS 20 | 37.00±5.26 | 14.58±3.03 | 24.25±1.88 | 70.21±11.28 | 146.03 |
| DS100 | 36.88±8.08 | 25.08±2.67 | 26.92±2.03 | 83.13± 2.77 | 172.01 |
| CM 5 | 37.63±5.96 | 13.33±2.36 | 22.30±9.83 | 100.63± 2.37 | 173.89 |
| CM 20 | 32.13±6.51 | 24.83±5.44 | 19.50±0.79 | 100.83± 3.66 | 177.29 |
| CM100 | 30.38±6.62 | 33.42±2.35 | 21.54±1.16 | 122.29± 5.47 | 207.63 |
| PM 5 | 18.04±4.76 | 19.63±4.95 | 20.17±1.17 | 81.25± 4.68 | 139.09 |
| PM 20 | 23.42±2.40 | 28.21±1.89 | 23.71±1.12 | 82.92± 1.56 | 158.26 |
| PM100 | 17.63±5.14 | 26.83±1.30 | 23.58±0.92 | 85.83± 2.00 | 153.87 |

However, significant differences ($p < 0.05$) were found among different wastes and different concentrations of wastes applied on the contents of exchangeable heavy metals.

Duncan's multiple range test was further applied to test the differences and the results are summarized in Table 5.5.

C Productivity and morphology of the harvested vegetables

a Productivity determined by the total fresh weight of plants harvested from various treatments of soils in acending order was activated sludge, digested sludge, chicken manure and pig manure (Fig. 5.1). For example, values were 2.7 gm from AS 100 soil, 6.7 gm from DS 100 soil, 9.0 gm from CM 100 soil and 15.3 gm from PM 100 soil. Therefore, animal manures were more superior as additives to soil for increasing productivity than sewage sludges. Digested sludge was better than activated sludge whereas pig manure was better than chicken manure. All of the treated soils showed better productivity than that of control, especially when the total oven-dried was compared.

b The morphological differences including the number of leaves and the percentage of the plants harvested which showed stage of maturity as flowering were shown in Table 5.6. Besides, the length of roots and shoots of the plants were also recorded (Fig. 5.2).

Table 5.5 Results of Duncan's multiple range test on the levels of exchangeable heavy metal content in the soils treated with various concentrations of wastes.

| Heavy metal | Waste effect | Concentration effect |
|---|---|---|
| Pb | 5 PM CM DS AS | NSD |
| | 20 PM CM DS AS | |
| | 100 PM CM DS AS | |
| Cu | NSD | AS 5 20 100 |
| | | DS 5 20 100 |
| | | CM 5 20 100 |
| | | PM 5 100 20 |
| Zn | NSD | NSD |
| Mn | 5 DS PM AS CM | AS 5 20 100 |
| | 20 DS PM AS CM | DS 5 20 100 |
| | 100 DS PM AS CM | CM 5 20 100 |
| | | PM 5 20 100 |
| Sum | 5 PM DS AS CM | AS 5 20 100 |
| | 20 DS PM CM AS | DS 5 20 100 |
| | 100 PM DS CM AS | CM 5 20 100 |
| | | PM 5 100 20 |

Arrangements were in an ascending order from left to right.

Items underlined with the same line denoted no significant difference ($p > 0.05$) between them.

NSD denoted no significant difference ($p > 0.05$) in two-way analysis of variance.

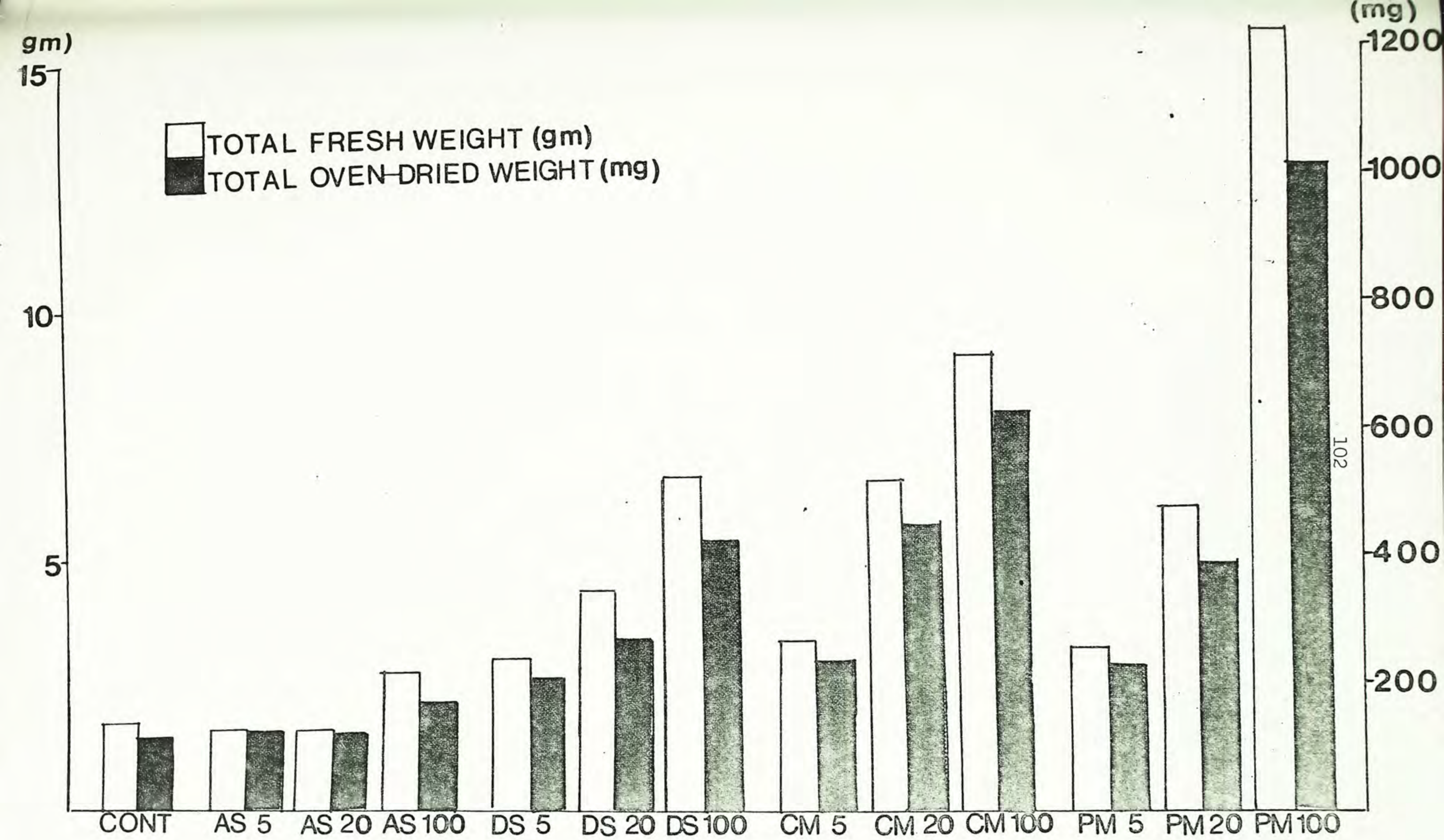


Fig. 5.1 Total fresh weight (gm) and total oven-dried weight (mg) of <u>Brassica</u> <u>parachinensis</u> harvested from soils treated with various concentrations of wastes

Table 5.6 Morphological difference of Brassica parachinensis harvested from soils treated with various concentrations of wastes.

| | Number of leaves | Percentage flowered |
|---|---|---|
| CONT | 5 | 0 |
| AS 5 | 6 - 7 | 0 |
| AS 20 | 5 - 8 | 25 |
| AS100 | 7 | 50 |
| DS 5 | 6 | 0 |
| DS 20 | 8 | 0 |
| DS100 | 6 - 13 | 0 |
| CM 5 | 3 - 8 | 0 |
| CM 20 | 8 - 9 | 0 |
| CM100 | 5 - 20 | 50 |
| PM 5 | 4 - 8 | 0 |
| PM 20 | 6 - 10 | 50 |
| PM100 | 13 - 29 | 100 |

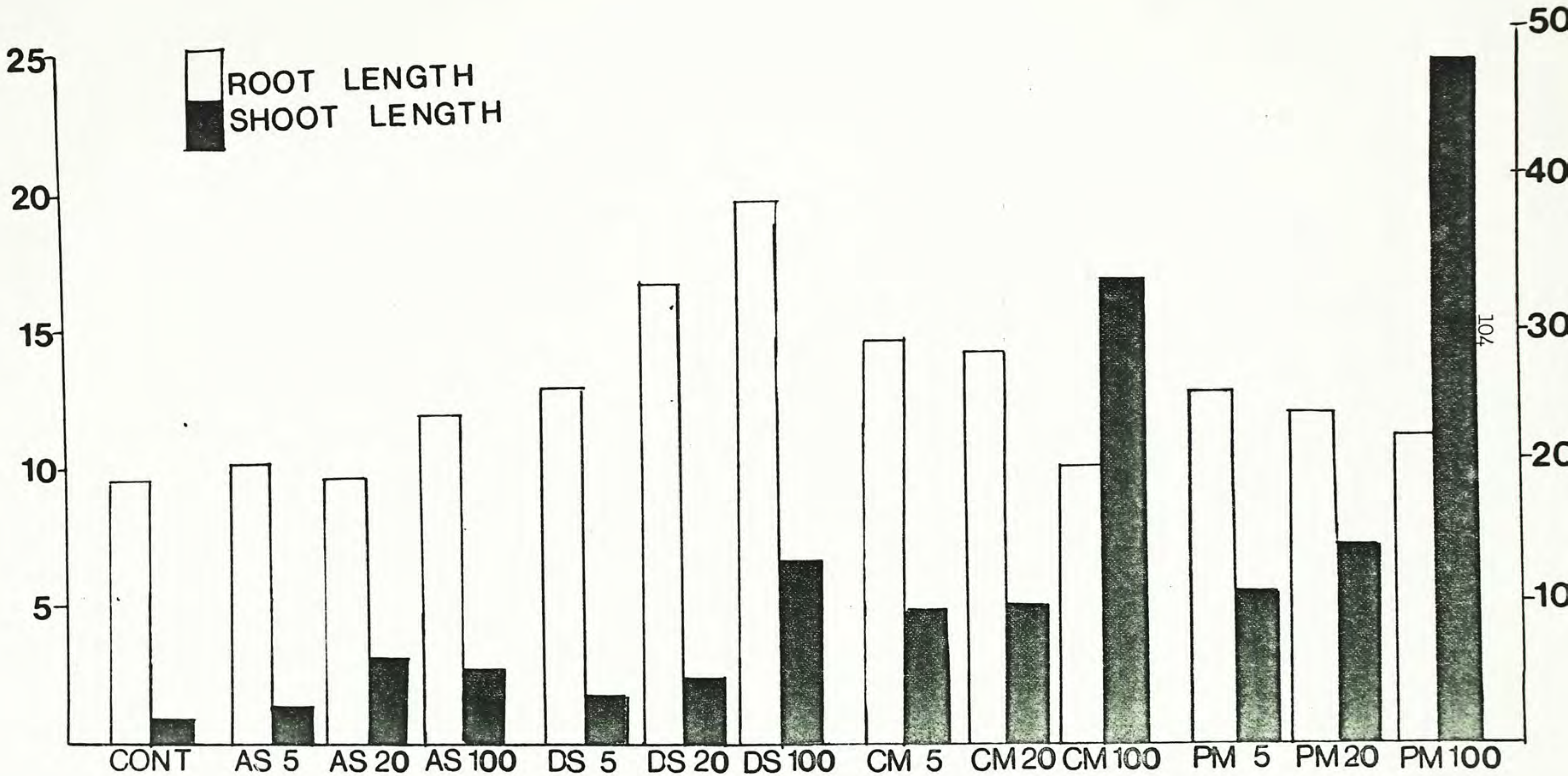


Fig. 5.2 Root length and shoot length (both in cm) of Brassica parachinensis harvested from soils treated with various concentrations of wastes

i Pig manure-amended soil had the highest number of leaves (29) followed by chicken manure-amended soil (20), digested sludge-amended soil (13) and activated sludge-amended soil (8). This was in close correlation with the productivity as expected (Table 5.6).

ii However, percentage of plants flowered should be considered together with shoot length of plants (Fig. 5.2). The plants from soils with activated sludge added were in dwarf forms. They were short, light and small compared with those from manured soil. Flowers of the plants were only in young budding stage compared with the long and mature flower stalks of the manure treated plants which were in a very ripen stage bearing seeds (Fig. 5.3 - 5.6).

D Heavy metal content of various parts of plants treated with various concentrations of wastes

The contents of Pb, Cu, Zn and Mn in different parts of plants harvested from soils treated with various concentrations of wastes are presented in Figs. 5.7 - 5.10. Same methods of statistical analysis (two-way analysis of variance followed by Duncan's multiple range test) were applied to process the data and the results are listed in Table 5.7.

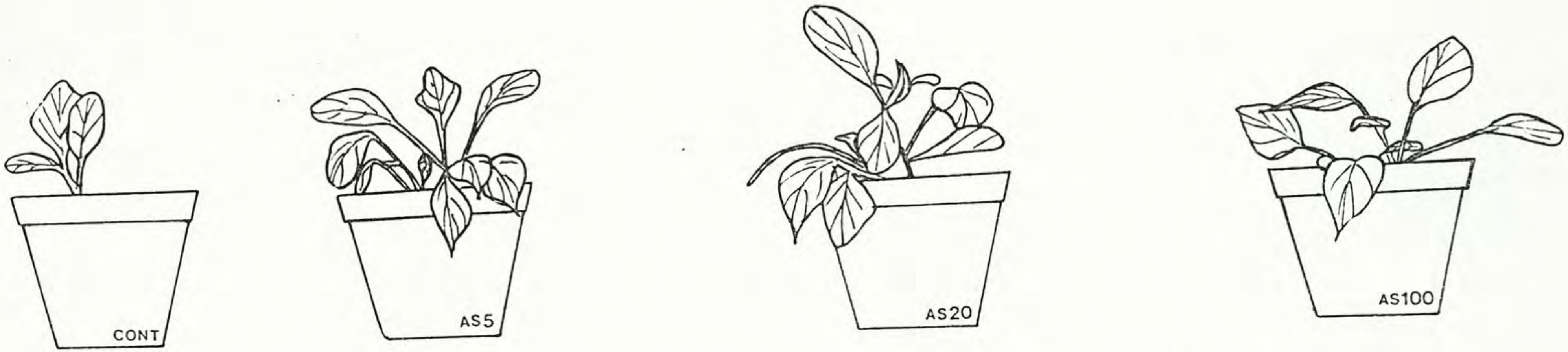


Fig. 5.3 Morphology of Brassica parachinensis harvested from soils treated with various concentrations of activated sludge

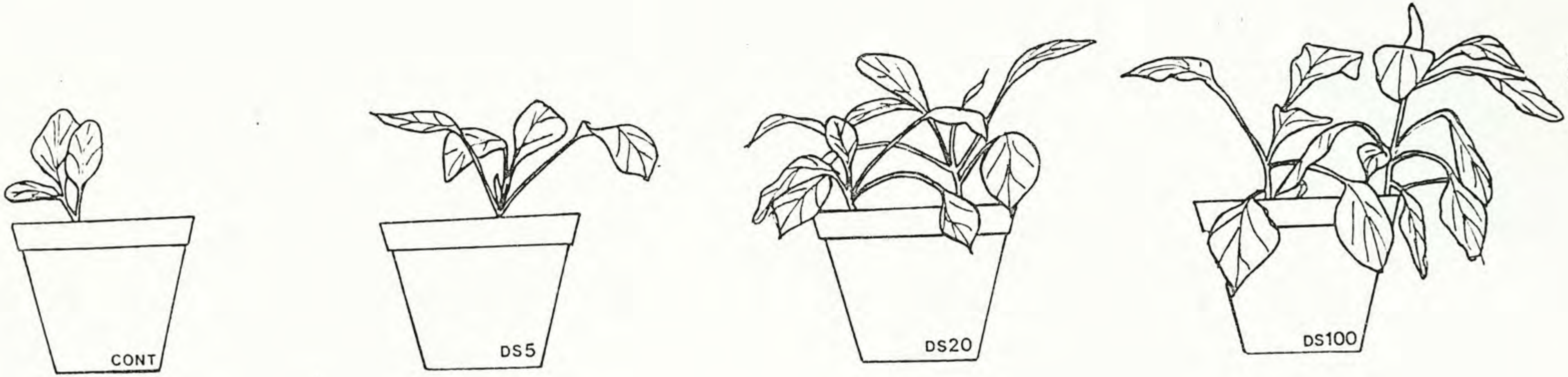


Fig. 5.4 Morphology of Brassica parachinensis harvested from soils treated with various concentrations of digested sludge


Fig. 5.5 Morphology of Brassica parachinensis harvested from soils treated with various concentrations of chicken manure


Fig. 5.6 Morphology of Brassica parachinensis harvested from soils treated with various concentrations of pig manure

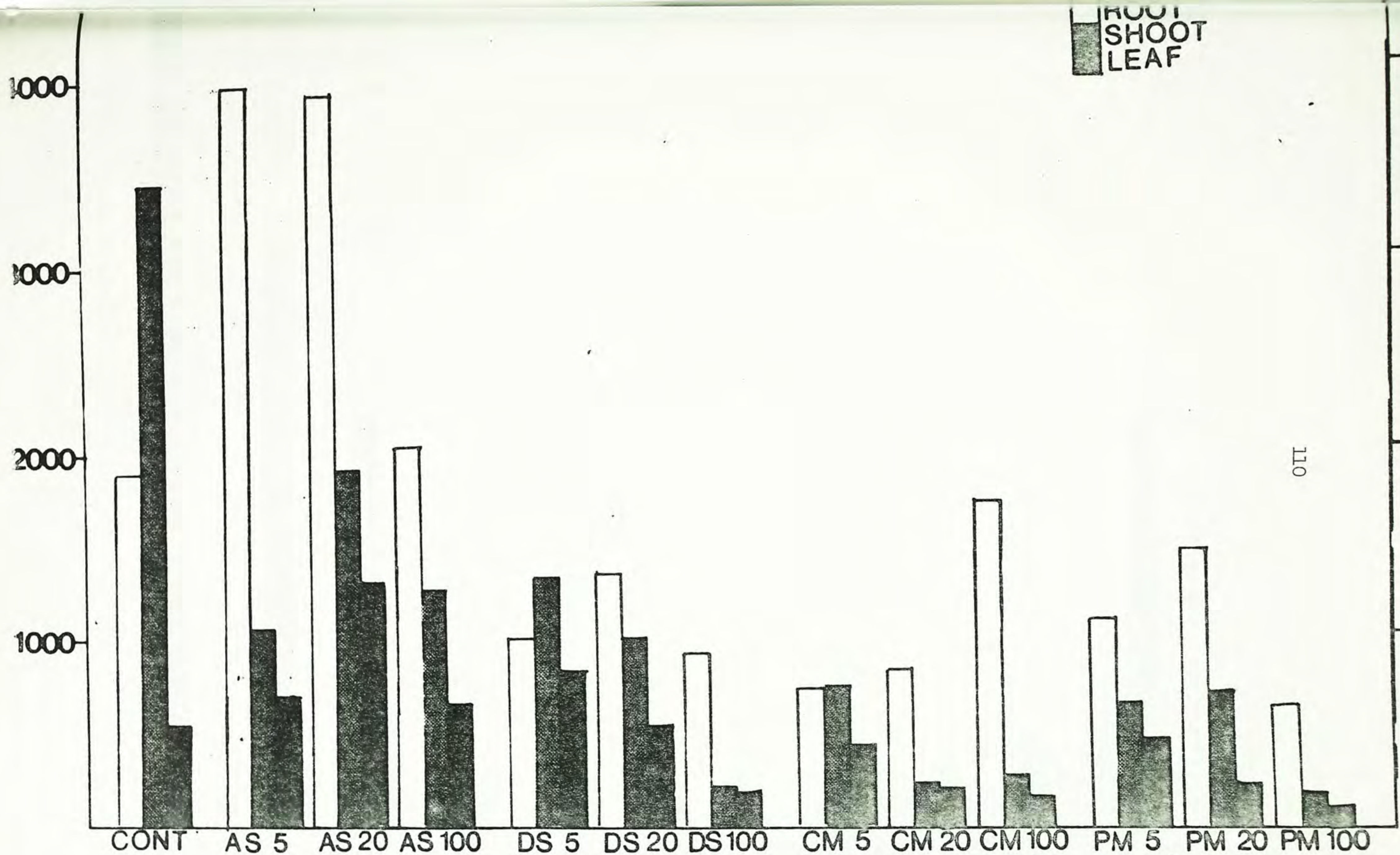


Fig. 5.7 Levels of lead content (ppm) in different parts of Brassica parachinensis harvested from soils treated with various concentrations of wastes

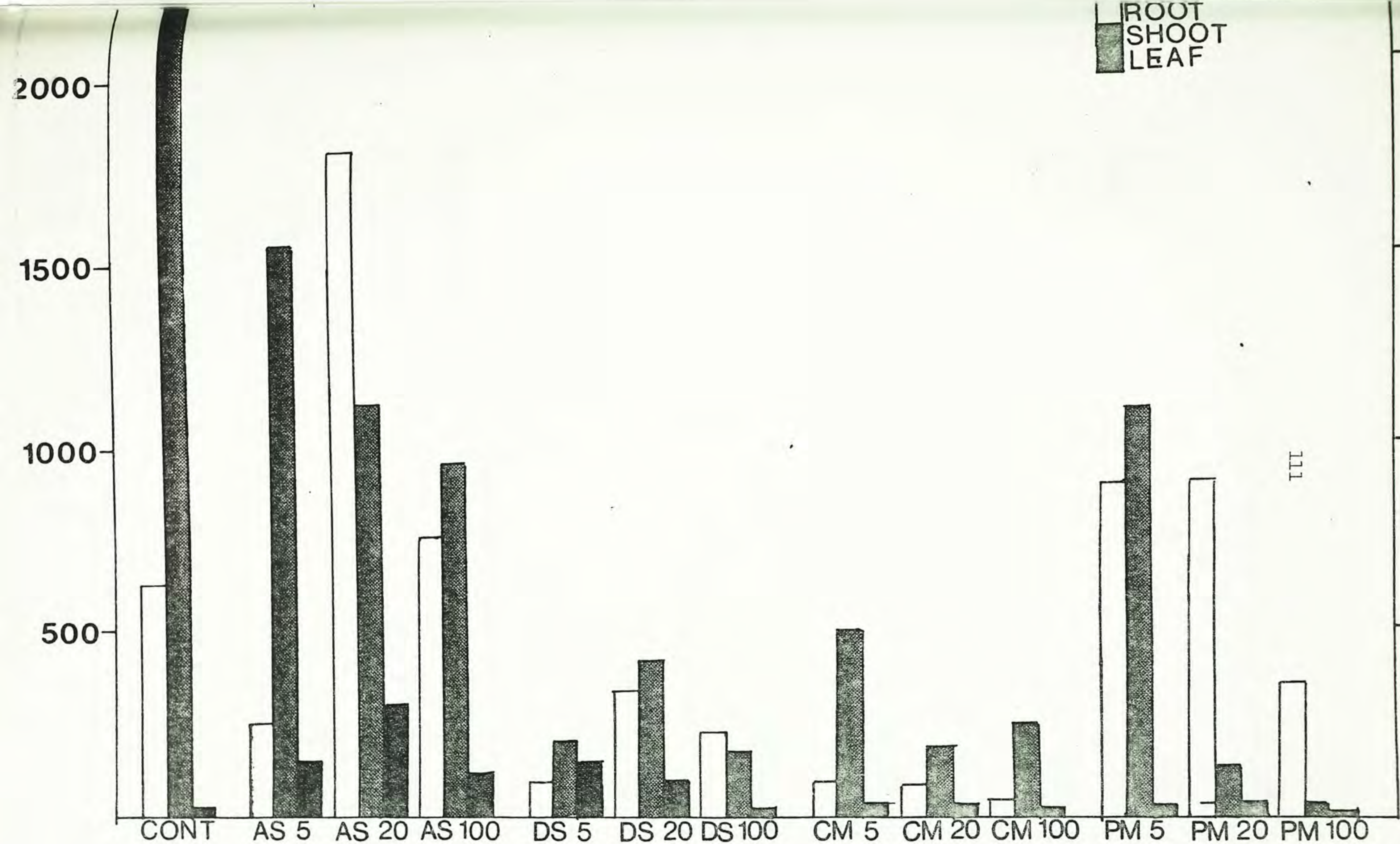


Fig. 5.8 Levels of copper content (ppm) in different parts of <u>Brassica parachinensis</u> harvested from soils treated with various concentrations of wastes

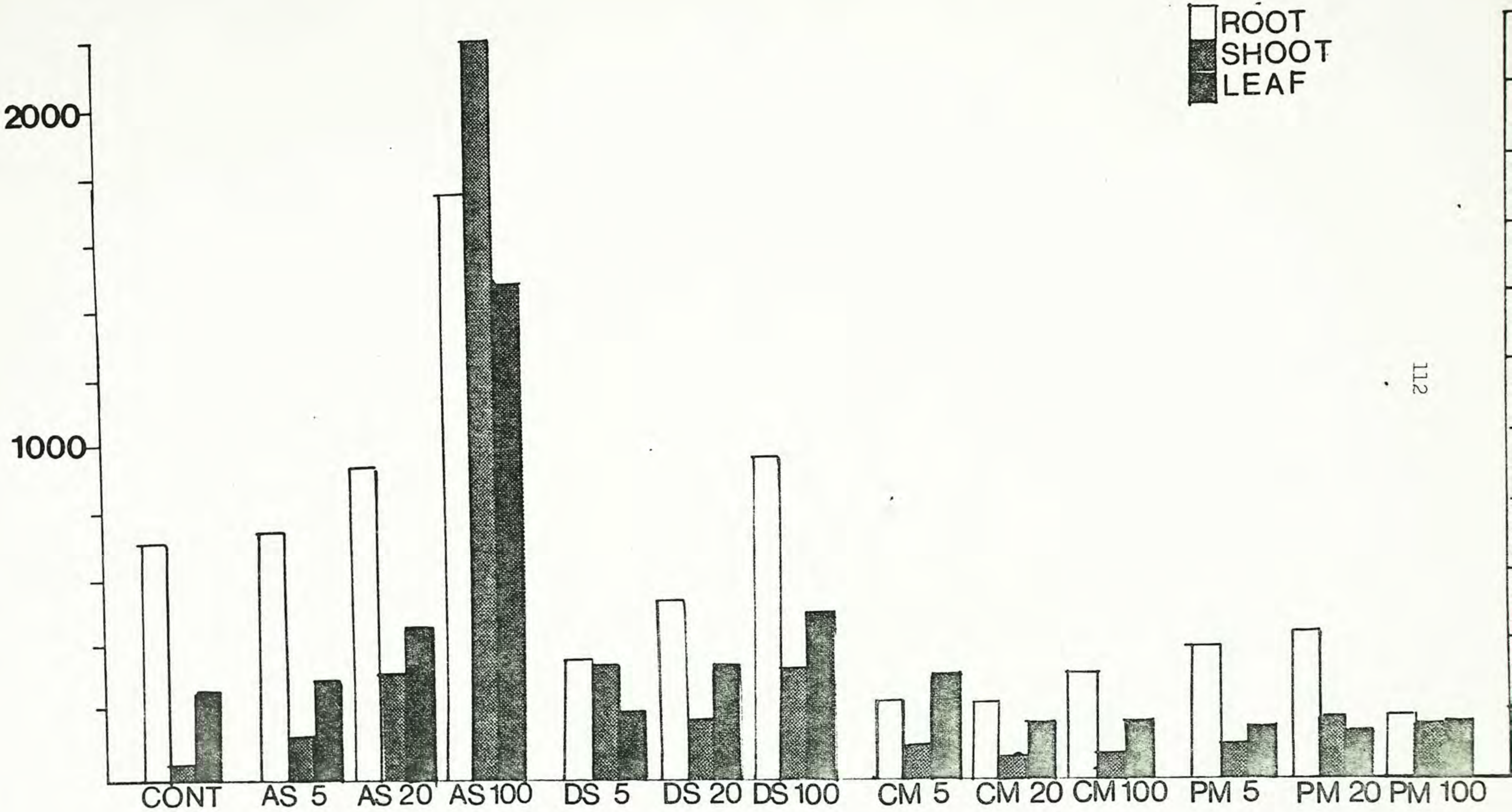


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Fig. 5.9 Levels of zinc content (ppm) in different parts of Brassica parachinensis harvested from soils treated with various concentrations of wastes

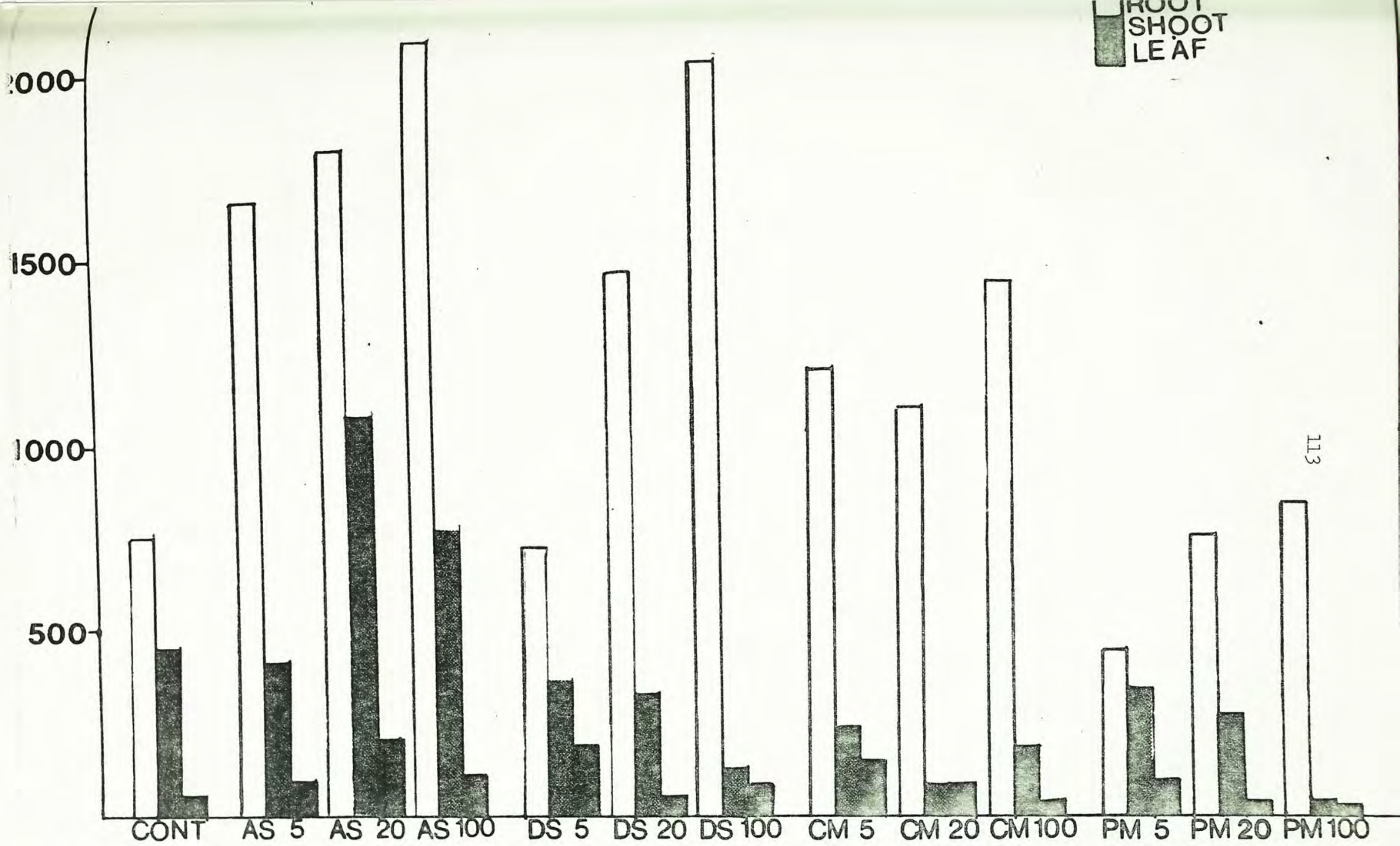


Fig. 5.10 Levels of manganese content (ppm) in different parts of Brassica parachinensis harvested from soils treated with various concentrations of wastes

Table 5.7 Results of Duncan's multiple range test on the levels of heavy metal content in different parts of plant harvested from soils treated with various concentration of wastes.

| Heavy metal | | Root | Shoot | Leaf |
|---|---|---|---|---|
| Pb | 5 | PM CM DS AS | | CM PM AS DS |
| | 20 | PM CM DS AS | NSD | PM CM DS AS |
| | 100 | PM CM DS AS | | PM CM DS AS |
| Cu | 5 | | DS CM PM AS | PM CM AS DS |
| | 20 | NSD | PM CM DS AS | PM CM DS AS |
| | 100 | | PM DS CM AS | PM CM DS AS |
| Zn | 5 | CM DS PM AS | | |
| | 20 | CM PM DS AS | NSD | NSD |
| | 100 | PM CM DS AS | | |
| Mn | 5 | PM DS CM AS | | |
| | 20 | PM CM DS AS | NSD | NSD |
| | 100 | PM CM DS AS | | |
| Sum | 5 | DS CM PM AS | CM PM DS AS | PM CM AS DS |
| | 20 | CM PM DS AS | CM PM DS AS | PM CM DS AS |
| | 100 | PM CM DS AS | PM CM DS AS | PM CM DS AS |

Arrangements were in an ascending order from left to right.
Items underlined with the same line denoted no significant difference ($p > 0.05$) between them.
NSD denoted no significant difference ($p > 0.05$) in two-way analysis of variance.

E Anatomical study on leaf sections

Symptoms of abnormalities in vegetables harvested from sewage sludge-amended soils had been revealed. Those harvested from manure-amended soils showed no abnormality with the same appearance as the control group. Symptoms of abnormalities included opaque granulate deposite and destruction of cells which were located at the lower epidermis of the activated sludge-grown leaves and the upper epidermis of the digested sludge-grown leaves (Figs. 5.12 and 5.13).

Appearance of symptoms was found to be dependent on the rates of application of wastes. Vegetables under AS 20, AS 100 and DS 100 treatments were found to have such symptoms while AS 5, DS 5 and DS 20 were not. This indicated that activated sludge was relatively more toxic than digested sludge in inducing anatomical abnormalities in leaf tissue.

## 5.4 DISCUSSION

Experiment results including morphology, final productivity, heavy metal content of plant tissue and anatomical studies of leaf sections indicated that animal manures were more preferable than sewage sludges for the use as a soil additive. In general, digested sludge was better than activated sludge while pig manure was better than chicken manure.

Relationships between the wastes (Cheung and Wong, 1981) and the waste amended-soils concerning their total and

Fig. 5.12 Deposition of opaque granules at cells of upper epidermal region of leaf harvested from DS100 treatment (300X)

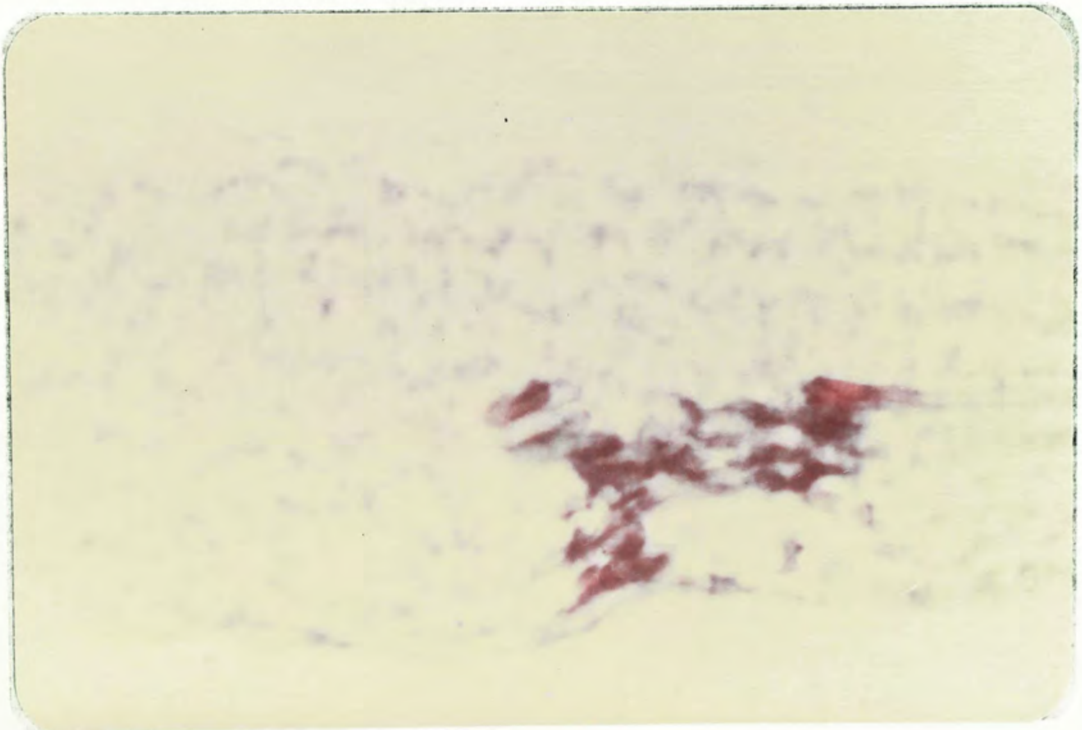

Fig. 5.12 Deposition of opaque granules at cells of lower epidermal region of leaf harvested from AS20 treatment (300X)

exchangeable heavy metal content were investigated (Section 2.3 B and Section 5.3 B of this thesis). No significant difference ($p > 0.05$) was found in the total heavy metal content among the four waste amended-soils (Table 5.8). Therefore, only exchangeable heavy metal contents of waste amended-soil could be compared with total heavy metal contents and exchangeable heavy metal contents of the wastes. In this investigation, only exchangeable Pb and Mn contents in waste-amended soils were found to be correlated with total pB and Mn contents rather than exchangeable Pb and Mn contents of the wastes (Table 5.8). Thus, a positive correlation between the levels of Pb and Mn in the wastes and the waste amended-soils was found.

The vegetables cultivated in activated sludge-amended soil incorporated the highest level of heavy metals, followed by those cultivated in digested sludge, chicken manure and pig manure-amended soil (Table 5.7). Such pattern did not exactly correlate with the metal contents in the wastes and waste-amended soils. This indicated that the availability of heavy metals to plants was not solely dependent on the concentration in the soils. Other edaphic conditions, such as soil pH and soil organic carbon levels could be determinating factors.

It was found that organic matter additions seemed to offer some promise as a method for the reduction of Pb uptake of plants which is probably related to the sorptive capacity of the organic matter (Zimdahl, 1979). Spearman's

Table 5.8 Patterns of difference in total and exchangeable heavy metal contents of wastes and waste-amended soils.

| Heavy metal | Total: Wastes | Total: Waste-amended soils | Exchangeable: Wastes | Exchangeable: Waste-amended soils |
|---|---|---|---|---|
| Pb | PM CM AS DS | NSD | AS CM DS PM | PM CM DS AS |
| Cu | AS PM CM DS | NSD | PM DS AS CM | NSD |
| Zn | AS CM PM DS | NSD | PM CM DS AS | NSD |
| Mn | AS DS PM CM | NSD | AS PM CM DS | DS PM AS CM |

Arrangements were in an ascending order from left to right.

Items underlined with the same line denoted no significant difference ($p > 0.05$) between them.

NSD denoted no significant difference ($p > 0.05$) in two-way analysis of variance.

rank correlation coefficients ($r_s$) were calculated in order to correlate the uptake of Pb in various parts ($r_s$ = -0.16 for root, -0.55 for shoot and -0.54 for leaf) of the vegetable with organic carbon of soils in this study.

In this experiment, sewage sludge-amended soils had lower soil pH and soil organic carbon than animal manure-amended soils (Table 5.2) which resulted in the accumulation of heavy metals in the crops. Similar results were also found by previous researchers. David and William (1979) found increase in Zn, Cu, Cd, Cr, Co and Pb in the surface soil which had been subjected to land and grass filtration of raw sewage. Accumulation of the heavy metals were also found in the herbage grown in such treated soil. It was also found that Cd of the sewage sludges possesses the tendency toward accumulation in cereal seed (Viitasalo, 1978). Among the three parts of the vegetable analysed, root portion had the highest level of Pb, Zn and Mn while leaf portion had the lowest level of Pb, Cu and Mn (Figs. 5.7 - 5.10). The sum of heavy metal content (Fig. 5.11) further demonstrated this pattern of uptake with root $>$ shoot $>$ leaf. Even though elevated levels of heavy metals resulted in plants grown from heavy metal enriched wastes, it was found that vegetative parts of plants remained relatively unaffected (Van Loon, 1974). In this study, the edible portion of Flowering Chinese Cabbage seemed to contain a lower level of heavy metals. However, precaution should be taken if vegetables

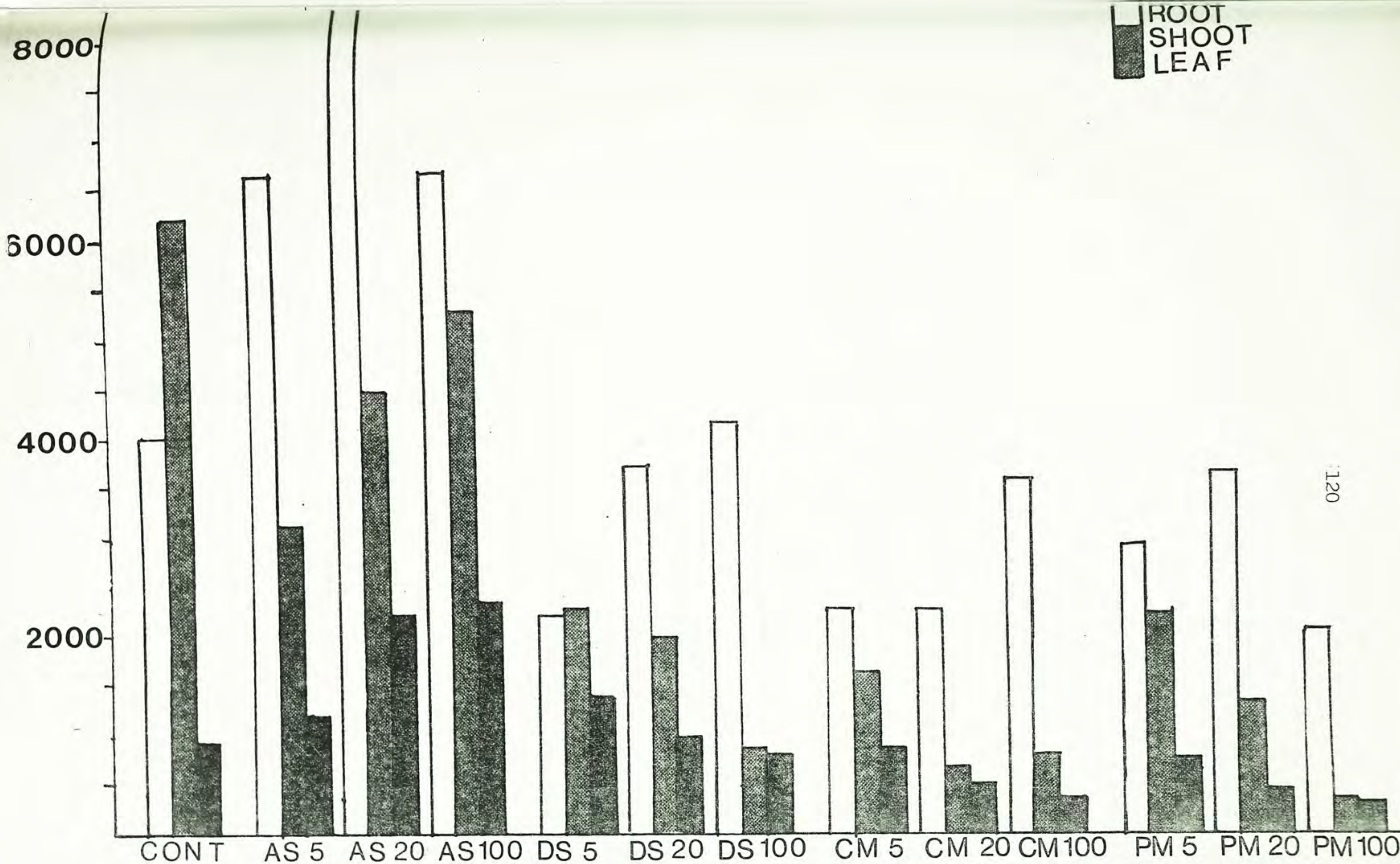


Fig. 5.11 Levels of sum heavy metal content (ppm) in different parts of Brassica parachinensis harvested from soils treated with various concentrations of wastes

like carrots are planted in waste-amended soil. The higher levels in the roots will be a health hazard.

Difference in the productivity of vegetables from various treated soils was found to be related to the properties of the wastes including contents of total nitrogen, organic carbon and extractable phosphate. The values of conductivity which reflected the salt levels by measuring the levels of cations in the soil also played an important role. Jeffery (1979) found that improvment of plant growth after application of piggery effluent to pastures was probably associated with increase in soil N, P and K and salt levels in the treated soils. Sewage sludge-amended soils had the lowest level of conductivity, organic carbon and extractable phosphate (Table 5.1) which contributed to the low productivity. On the other hand, animal manure-amended soils had higher levels of soil pH, conductivity organic carbon and extractable phosphate. Such edaphic conditions decreased the availability of the heavy metals to plants.

According to the results of the present experiment, it can be concluded that animal manures are better soil additives than sewage sludges for the growth of Flowering Chinese Cabbage. However, adverse effects on soils, plants and water could be detected from over-manured areas and surrounding area of concentrated animal production (Bassam and Thorman, 1979). The main adverse effect resulted from high application rates were the eutrophication of receiving waters and the deterioration of plant quality results from nitrate accumulation. Thus, proper monitoring of these wastes will be essential if they were applied to agricultural lands in a large scale.

# CHAPTER SIX

## Heavy metal transfer in a simulated, aquatic, two-level food chain (Chlorella pyrenoidosa and Palaeomonetes sp.)

### 6.1 INTRODUCTION

Part one of this thesis, Chapters 3 - 5, reported the studies on the utilization of animal manures and sewage sludges for biomass production in form of alga, fish and vegetable. Heavy metals were found to be accumulated from the wastes to the harvested products. Nevertheless, the subsequent effects of accumulated heavy metals in primary producers on organisms at higher trophic levels were found heterogenous. Both accumulation and elimination were noted.

Accumulation of heavy metals through food chain was found by various authors. When sea worms were used to feed crustaceans, mercury (Hg) was found accumulated in the higher trophic level (Aubert et al., 1976). In a food chain study involving phytoplankton (Dunaliella marina), zooplankton (Artemia salina) and shrimp (Lysmata seticaudata), efficient transfer of organic arsenic (As) was found (Wrench et al., 1979). In another food chain comprising plankton (Navicula sp.), mollusk (Scorbicularoa sp.) and crab (Carcinus sp.), magnification of cobalt (Co) was demonstrated (Amiard and Amiard, 1975). Same sort of food chain study comprised heterotrophic bacteria, tubificid worm and fish showed the accumulation of Pb, Cu, Zn, Mn, Fe and Cr (Patrick and Loutit, 1976, 1978).

When discussing accumulation in living organisms, two more specific terms should be introduced :

Bioaccumulation is the ability of an organism to concentrate the element above environmental levels.

Biomagnification is the tendency for elements to be concentrated with trophic level transfer.

Some authors held opposite opinions to those who supported biomagnification. They claimed that only primary producer level of the food chains was affected by the heavy metal concentrations in the environment. Primary consumer and higher trophic level organisms were not affected. In a food chain comprised of alga (*Croomonas salina*) and calcanoid copepod (*Acartia tonsa*), Hg was found only accumulated in alga probably by surface sortion but not transferred to the copepod (Parrish and Carr, 1976). Hg was also found not to be transferred through nearshore phytoplankton and zooplankton to anchovies (Knauer and Martin, 1972).

Such elimination of incorporated heavy metals was carried out by various means. Zooplankton was found able to eliminate heavy metal in phytoplankton food in its faecal pellets (Marshall et al., 1975). Other mechanisms also played a role in elimination of the incorporated heavy metals. Exoskeletons of shrimps were found accumulated with heavy metals which were shed off when molting occurred (Bertine and Goldberg, 1972).

In this experiment, an aquatic food chain was simulated in order to investigate the phenomena of biomagnification and bioelimination. The simulated food chain comprised various wastes as the nutrient level, green alga, Chlorella pyrenoidosa, as primary producer level and freshwater shrimp, Palaeomonetes sp., as primary consumer. Algal products harvested from waste suspensions were used to feed freshwater shrimps. Heavy metal contents of both the producer and consumer levels were compared.

## 6.2 MATERIALS AND METHODS

### A Collection and treatment of samples of sewage sludges and animal manure

The sewage sludge samples were obtained from two sites : activated sludge from the sewage treatment plant at The Chinese University and digested sludge from Shek Wu Hui Sewage Treatment Plant. The animal manures samples including chicken manure and pig manure were collected from farms in the New Territories. Both samples were dried under the sun for two weeks and grinded by a hand-driven grinder. They were then passed through a 2 mm sieve before use.

### B Preparation of media for cultivation of algae

After a preliminary study, 1 and 2% (w/v) of the waste materials were chosen for algae culture. Extraction of the sewage sludges or animal manure was by means of autoclaving for 20 minutes followed by suction filtration through Whatman No. 42 filter papers (Wong, 1980). Bristol medium (Starr,

1960) was used as a control medium for comparison.

C Cultivation of algae in various media

A volume of 1.5 litre of each medium was placed in a 2 litre conical flask. Cotton wool was packed at the mouth of the flask and a 10 ml pipette was inserted in the middle of the cotton sool to serve as an air inlet. The whole sets of apparatus containing various media were autoclaved again for another 20 minutes before the inoculation of algae. 100 ml of Chlorella pyrenoidosa suspension (concentration : $5.97 \times 10^6$ cells per ml) was inoculated into each container. The flasks were then placed on illuminated benches for 11 days at $25^{\circ}C \pm 2^{\circ}C$ and 70 - 80% relative humidity with 16 hr. light (2,500 lux) - 8 hr. dark cycle. Compressed air after filtering through water was used to aerate the media during the culture period.

D Analysis of properties of waste-grown algae

The number of algal cells was recorded at the end of the culture period using a Neübauer counting chamber. Oven-dried weight (at $105^{\circ}C$ for 24 hr.), chlorophyll content (Arnon, 1949), protein content (Lowry, 1951) and carbohydrate content (Dreywood, 1946) of the algal products were tested at the end of the culture period. Phosphate content (Watanabe and Olsen, 1962) and heavy metal contents (Pb, Cu, Zn and Mn) were also measured after the dry ashing method (Allen et al., 1974).

E Harvesting method for algae

Algal cells were harvested at the end of the culture period. Algal suspensions were centrifuged for 15 minutes at 5,00 rpm by a Sorvall Rc 2-B Automatic Refrigerated Centrifuge and then washed twice by distilled water and followed by another centrifugation. Finally, cells were suspended in about 200 ml distilled water and kept in the refrigerator as feeding materials.

F Rearing freshwater shrimps with various harvested algal products

Transparent plastic aquaria measuring 18 x 8 x 10 $cm^3$ were used as culture vessels. Water was filled to two-third of the aquaria and filtered compressed air was bubbled through airstones. Six shrimps were placed in each aquarium at the beginning of the culture period. 10 ml of concentrated algal suspension was added to each aquarium and freshwater was replaced every day during the two-week culture period. Moulted exoskeletons from the shrimps were collected for the analysis of heavy metals.

G Analysis of harvested shrimps

Body weights of the shrimps were measured both before and after the culture. Harvested shrimps were killed and divided into meat and exoskeleton portions. Heavy metal contents (Pb, Cu, Zn and Mn) of meat and exoskeleton portions of the shrimps and also the moulted exoskeleton during culture were measured by an atomic absorption spectrophotometer after the dry ashing method (Allen et al., 1974).

## 6.3 RESULTS

A A preliminary study was conducted in order to compare the algal products harvested from various concentrations of waste extracts. 3 and 6% media were prepared for the culture. However, such concentrations of manure extracts were dark brown in color and had a high turbidity, thus algal growth was inhibited. Final analysis of algal products could only be conducted on those harvested from sewage sludge extracts and the results were listed in Tables 6.1 to 6.3. Experimental results showed that activated sludge extracts inhibited growth of Chlorella pyrenoidosa when compared with Bristol medium. On the other hand, digested sludge extracts supported a better growth. This coincided with that found in the previous study (Section 3.3 of this thesis). When compared with Bristol medium, significantly higher cell number ($p < 0.01$), oven-dried weight ($p < 0.01$), and contents of chlorophyll ($p < 0.01$), protein ($p < 0.01$) and carbohydrate ($p < 0.05$) were found in suspensions of digested sludge. On the other hand, suspensions of activated sludge had significantly lower cell count ($p < 0.01$), oven-dried weight ($p < 0.01$), and contents of chlorophyll ($p < 0.01$), protein ($p < 0.05$) and carbohydrate ($p < 0.05$). However, phosphate content was found significantly lower ($p < 0.01$) in activated

Table 6.1 Cell count, oven-dried weight and chlorophyll content of algal suspensions at the end of the culture period.

| | Cell count ($x10^6$/ml) | Oven-dried weight (mg/l) | Chlorophyll content (µg/ml) |
|---|---|---|---|
| Bristol medium | 2.12±0.26 | 263.16±24.44 | 1.01±0.13 |
| Activated sludge | | | |
| 1% extract | 1.54±0.17*** | 222.73± 7.29*** | 0.79±0.08*** |
| 2% extract | 1.37±0.16*** | 228.54±10.37*** | 0.99±0.09 |
| Digested sludge | | | |
| 1% extract | 3.45±0.39*** | 409.42±15.38*** | 2.74±0.22*** |
| 2% extract | 4.84±0.52*** | 571.69±17.43*** | 3.46±0.41*** |

* significant different ($p < 0.10$) from Bristol medium

** significant different ($p < 0.05$) from Bristol medium

*** significant different ($p < 0.01$) from Bristol medium

Table 6.2 Nutrient contents of algal products harvested from Bristol medium and various sludge extracts.

| | Protein (mg/l) | Carbohydrate (mg/l) | Phosphate (ppm) |
|---|---|---|---|
| Bristol medium | 129.58 ± 6.68 | 20.34 ± 2.42 | 13,889 ± 604 |
| Activated sludge | | | |
| 3% extract | 60.42 ± 5.88*** | 12.53 ± 5.74*** | 7,642 ± 213*** |
| 6% extract | 117.15 ± 9.86** | 13.69 ± 6.41** | 12,218 ± 519*** |
| Digested sludge | | | |
| 3% extract | 174.03 ± 13.01*** | 40.75 ± 9.36** | 14,967 ± 721*** |
| 6% extract | 232.26 ± 19.31*** | 50.84 ± 8.49*** | 15,482 ± 852*** |

* significant different ($p < 0.10$) from Bristol medium

** significant different ($p < 0.05$) from Bristol medium

*** significant different ($p < 0.01$) from Bristol medium

Table 6.3 Heavy metal contents (ppm) of algal products harvested from Bristol medium and various sludge extracts.

| | Pb | Cu | Zn | Mn |
|---|---|---|---|---|
| Bristol medium | 65.71± 3.58 | 784.35± 68.42 | 952.06± 52.43 | 147.25± 6.49 |
| Activated sludge | | | | |
| 3% extract | 223.49±11.27*** | 2,850.62±156.74*** | 4,237.29±157.62*** | 475.70± 9.54*** |
| 6% extract | 305.13± 8.67*** | 3,941.54±170.23*** | 5,529.46±163.94*** | 521.51±13.53*** |
| Digested sludge | | | | |
| 3% extract | 79.65± 4.82*** | 1.042.48± 64.70*** | 1,329.21±130.42*** | 175.69±18.82*** |
| 6% extract | 84.92± 7.53*** | 1,467.84±153.26*** | 1,694.40±200.67*** | 226.38±25.71*** |

* significant different ($p < 0.10$) from Bristol medium
** significant different ($p < 0.05$) from Bristol medium
*** significant different ($p < 0.01$) from Bristol medium

sludge suspensions and higher ($p < 0.01$) in digested sludge suspensions. Furthermore, algal products from sewage sludge media accumulated significantly higher ($p < 0.01$) levels of heavy metals than that from Bristol medium. However, digested sludge-grown algal product accumulated comparatively lower heavy metal levels.

B In order to compare the algal products, same concentrations of sewage sludges and animal manures (1 and 2% w/v) of the wastes were prepared for algal culture. Final analysis of algal suspensions (Tables 6.4 and 6.5) and also heavy metal contents of algal products (Table 6.6 and Fig. 6.1) are listed.

The difference between animal manures and sewage sludges was noted in the results of algal products harvested from the waste suspensions. Animal manures extracts were more suitable for algal growth than sewage sludge extracts. This was reflected by the properties of algal suspensions (cell count, oven-dried weight, contents of chlorophyll, protein and carbohydrate) and algal products (phosphate and heavy metals including Pb, Cu, Zn and Mn) (see also Section 3.3 of the thesis). Activated sludge algal suspensions had significantly lower cell count ($p < 0.05$), oven-dried weight ($p < 0.01$) and contents of chlorophyll ($p < 0.01$), protein ($p < 0.01$) and carbohydrate ($p < 0.01$). Algal products from activated sludge media had significantly lower ($p < 0.01$) phosphate content but higher ($p < 0.01$) level of heavy metal contents. Pig manure algal suspensions had significantly

Table 6.4 Cell count, oven-dried weight and chlorophyll content of algal suspensions at the end of the culture period.

| | Cell count ($x10^6$/ml) | Oven-dried weight (mg/l) | Chlorophyll content (µg/ml) |
|---|---|---|---|
| Bristol medium | 2.67±0.32 | 320.29±12.20 | 36.50±3.83 |
| Activated sludge | | | |
| 1% extract | 2.06±0.21*** | 284.43±16.58*** | 28.71±3.15*** |
| 2% extract | 2.39±0.23* | 257.60±16.36*** | 26.54±2.98*** |
| Digested sludge | | | |
| 1% extract | 2.59±0.29 | 367.54±21.37*** | 52.00±5.40*** |
| 2% extract | 3.13±0.24*** | 427.56±41.79*** | 56.72±5.87*** |
| Chicken manure | | | |
| 1% extract | 3.98±0.43*** | 482.32±44.65*** | 77.48±7.99*** |
| 2% extract | 4.67±0.39*** | 487.17±51.63*** | 78.75±7.49*** |
| Pig manure | | | |
| 1% extract | 5.58±0.42*** | 590.92±65.84*** | 83.20±8.17*** |
| 2% extract | 6.21±0.47*** | 600.82±63.63*** | 89.25±9.13*** |

* significant different ($p < 0.10$) from Bristol medium

** significant different ($p < 0.05$) from Bristol medium

*** significant different ($p < 0.01$) from Bristol medium

Table 6.5 Nutrient contents of algal products harvested from Bristol medium and various waste extracts.

| | Protein (mg/l) | Carbohydrate (mg/l) | Phosphate (ppm) |
|---|---|---|---|
| Bristol medium | $123.67 \pm 4.65$ | $23.90 \pm 2.42$ | $14,453 \pm 760$ |
| Activated sludge | | | |
| 1% extract | $35.45 \pm 4.79^{***}$ | $11.60 \pm 0.98^{***}$ | $8,350 \pm 467^{***}$ |
| 2% extract | $41.80 \pm 2.20^{***}$ | $14.75 \pm 1.67^{***}$ | $11,562 \pm 638^{***}$ |
| Digested sludge | | | |
| 1% extract | $161.60 \pm 4.79^{***}$ | $34.75 \pm 3.92^{***}$ | $15,360 \pm 852^{*}$ |
| 2% extract | $171.80 \pm 12.20^{***}$ | $52.05 \pm 5.49^{***}$ | $17,251 \pm 958^{***}$ |
| Chicken manure | | | |
| 1% extract | $201.45 \pm 5.80^{***}$ | $71.75 \pm 7.57^{***}$ | $29,166 \pm 1,486^{***}$ |
| 2% extract | $250.00 \pm 16.36$ | $77.40 \pm 6.53^{***}$ | $33,635 \pm 1,657^{***}$ |
| Pig manure | | | |
| 1% extract | $223.58 \pm 29.59^{***}$ | $83.20 \pm 7.68^{***}$ | $50,256 \pm 2,021^{***}$ |
| 2% extract | $303.93 \pm 2.54^{***}$ | $89.25 \pm 9.02^{***}$ | $51,206 \pm 2,124^{***}$ |

* significant different ($p < 0.10$) from Bristol medium

** significant different ($p < 0.05$) from Bristol medium

*** significant different ($p < 0.01$) from Bristol medium

Table 6.6 Heavy metal contents (ppm) of algal products harvested from Bristol medium and various waste extracts.

| | Pb | Cu | Zn | Mn |
|---|---|---|---|---|
| Bristol medium | 72.35± 9.10 | 731.16±34.67 | 984.36± 89.42 | 195.94± 8.72 |
| Activated sludge | | | | |
| 1% extract | 154.60±11.38*** | 1,675.46±70.35*** | 2,630.38±132.37*** | 247.64±17.69*** |
| 2% extract | 217.42±19.81*** | 2,403.37±85.48*** | 3,054.71±210.68*** | 360.17±20.14*** |
| Digested sludge | | | | |
| 1% extract | 39.19± 5.49*** | 692.25±74.92 | 751.24± 69.54*** | 97.58±10.05*** |
| 2% extract | 60.98± 7.12** | 850.59±79.57*** | 942.37± 74.26 | 145.15±11.28*** |
| Chicken manure | | | | |
| 1% extract | 46.65± 6.27*** | 382.92±27.39*** | 562.54± 63.90*** | 227.42±22.19*** |
| 2% extract | 49.28± 4.08*** | 495.62±56.42*** | 723.82± 64.87*** | 334.43±29.36*** |
| Pig manure | | | | |
| 1% extract | 63.67± 5.92* | 319.02±10.18*** | 346.39± 42.02*** | 123.02±15.48*** |
| 2% extract | 72.74± 9.67 | 360.17±18.67*** | 497.30± 48.79*** | 172.29±10.67*** |

* significant different ($p < 0.10$) from Bristol medium

** significant different ($p < 0.05$) from Bristol medium

*** significant different ($p < 0.01$) from Bristol medium

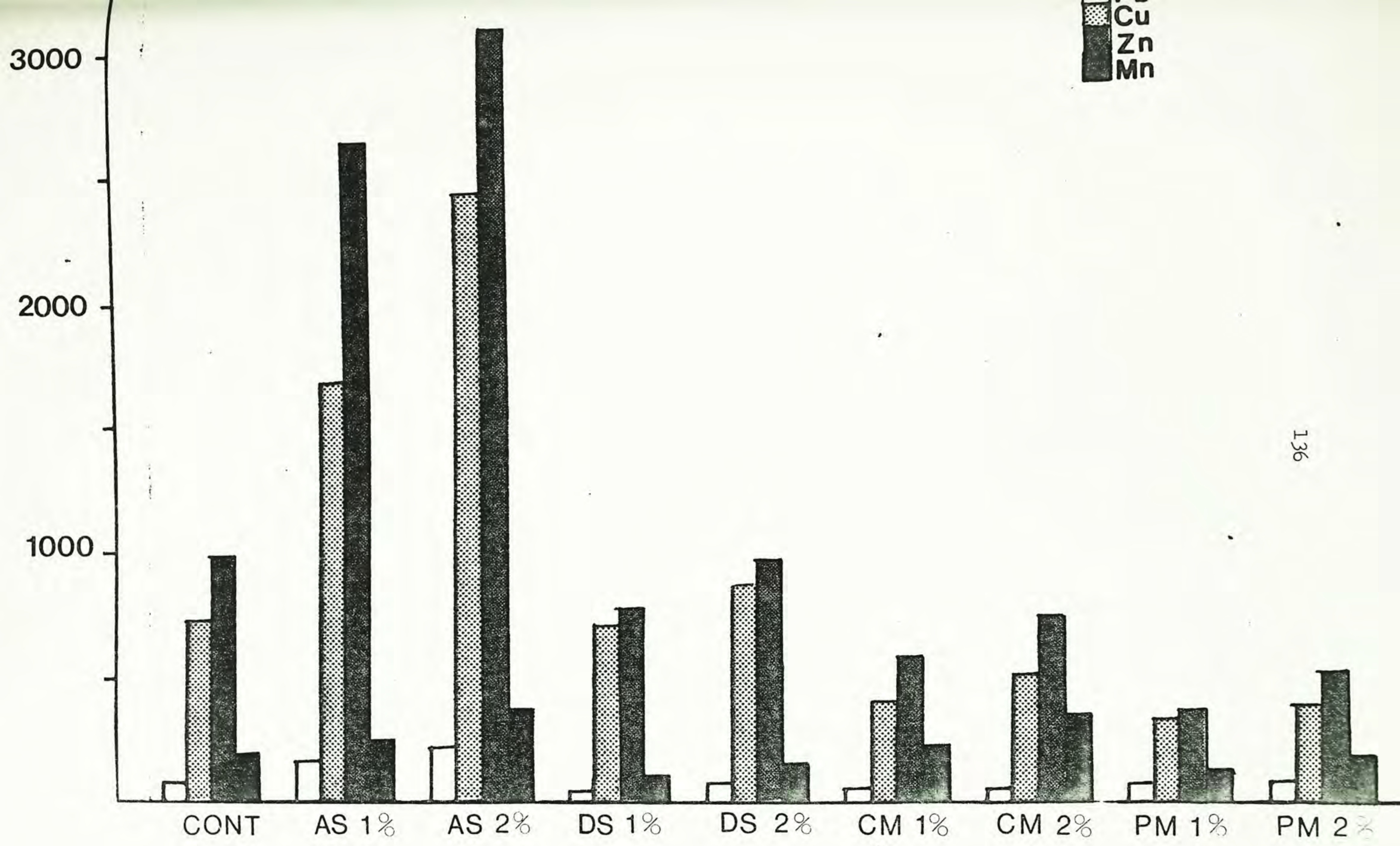


Fig. 6.1 Heavy metal contents (ppm) of algal products harvested from various concentrations of wastes

higher cell count ($p < 0.01$), oven-dried weight ($p < 0.01$) and contents of chlorophyll ($p < 0.01$), protein ($p < 0.01$) and carbohydrate ($p < 0.01$). Algal products from pig manure media had significantly higher ($p < 0.01$) phosphate content but significantly lower ($p < 0.01$) level of heavy metal contents. Digested sludge was found relatively better than activated sludge while chicken manure was inferior when compared with pig manure.

C The results of the changes in body weight, the heavy metal contents of meat and exoskeletons of shrimps and moulted exoskeletons of shrimps during the culture period are listed in Tables 6.7 to 6.10 and Figs. 6.2 to 6.4. The increase of body weight of shrimps when harvest ranged from 6.19 to 44.50%. The highest increase was found in pig manure extracts, followed by chicken manure extracts and digested sludge extracts whereas the lowest increase in activated sludge extracts. Activated sludge was the only waste which showed inferior growth when compared with the Bristol medium (Table 6.7).

The aim of analysising the heavy metal contents of the meat and exoskeleton portions of the shrimps harvested and the moulted exoskeletons during the culture period was to investigate any incidence of biomagnification and bioelimination of such heavy metals. The pattern of difference in heavy metal contents was found to be moulted exoskeleton > harvested

Table 6.7 Percentage increase in body weight of shrimps fed with various waste grown algal products.

| | Average body weight before cultivation (mg) | Average body weight at harvest (mg) | Increase in body weight (%) |
|---|---|---|---|
| Bristol medium | 200.12 | 224.73 | 12.30±1.50 |
| Activated sludge | | | |
| 1% extract | 201.75 | 214.24 | 6.19±0.80*** |
| 2% extract | 191.18 | 205.59 | 7.54±0.90*** |
| Digested sludge | | | |
| 1% extract | 219.35 | 257.21 | 17.26±1.80*** |
| 2% extract | 190.67 | 234.58 | 23.03±2.40*** |
| Chicken manure | | | |
| 1% extract | 278.82 | 378.86 | 35.88±3.90*** |
| 2% extract | 211.36 | 293.16 | 38.70±4.20*** |
| Pig manure | | | |
| 1% extract | 206.93 | 293.01 | 41.60±4.30*** |
| 2% extract | 230.90 | 333.65 | 44.50±4.70*** |

* significant different ($p > 0.10$) from Bristol medium

** significant different ($p > 0.05$) from Bristol medium

*** significant different ($p > 0.01$) from Bristol medium

Table 6.8 Heavy metal contents (ppm) of meat of shrimps fed with various waste grown algal products.

| | Pb | Cu | Zn | Mn |
|---|---|---|---|---|
| Bristol medium | 69.28± 7.24 | 716.31± 72.47 | 963.64± 99.14 | 218.92±24.23 |
| Activated sludge | | | | |
| 1% extract | 182.31±19.75*** | 1.825.36±191.87*** | 2,478.99±279.35*** | 570.28±58.62*** |
| 2% extract | 235.14±22.22*** | 3,423.81±359.61*** | 3,320.82±334.82*** | 685.05±75.43*** |
| Digested sludge | | | | |
| 1% extract | 119.27±13.69*** | 1,198.63±125.60*** | 1,771.28±189.44*** | 343.37±42.58*** |
| 2% extract | 128.77±13.48*** | 1,426.39±179.02*** | 1,914.18±217.96*** | 374.71±47.12*** |
| Chicken manure | | | | |
| 1% extract | 95.87±10.31*** | 951.23±111.78*** | 1,332.16±144.25*** | 294.24±35.75*** |
| 2% extract | 94.57±11.53*** | 961.54±113.65*** | 1,350.88±165.71*** | 305.38±36.23*** |
| Pig manure | | | | |
| 1% extract | 78.14± 9.88* | 742.96± 77.58 | 1.056.70±113.51 | 239.62±27.71 |
| 2% extract | 85.06± 8.61*** | 839.24± 95.35** | 1,204.70±141.57*** | 272.57±24.59*** |

* significant different ($p > 0.10$) from Bristol medium

** significant different ($p > 0.05$) from Bristol medium

*** significant different ($p > 0.01$) from Bristol medium

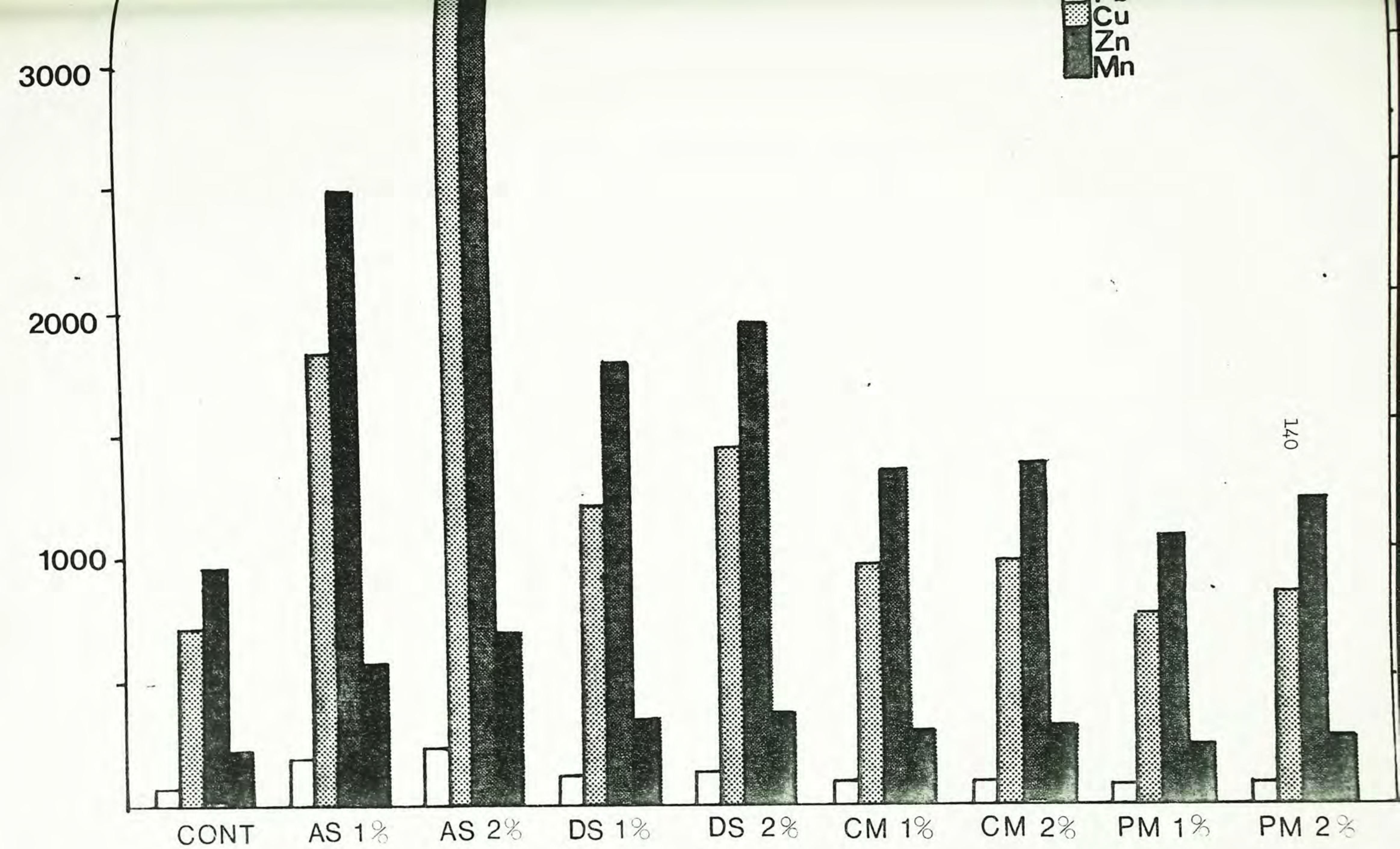


Fig. 6.2 Heavy metal contents (ppm) of harvested meat of shrimps fed with various waste-grown algae

Table 6.9 Heavy metal contents (ppm) of exoskeletons of shrimps fed with various waste grown algal products.

| | Pb | Cu | Zn | Mn |
|---|---|---|---|---|
| Bristol medium | 112.42 ± 9.33 | 1,092.73 ± 110.41 | 1,532.89 ± 152.73 | 344.22 ± 25.36 |
| Activated sludge | | | | |
| 1% extract | 182.41 ± 15.36$^{***}$ | 2,015.91 ± 213.24$^{***}$ | 2,847.20 ± 269.21$^{***}$ | 562.54 ± 47.21$^{***}$ |
| 2% extract | 237.14 ± 21.69$^{***}$ | 2,367.22 ± 250.26$^{***}$ | 3,280.49 ± 358.24$^{***}$ | 718.10 ± 81.86$^{***}$ |
| Digested sludge | | | | |
| 1% extract | 169.53 ± 15.64$^{***}$ | 1,074.28 ± 112.91 | 1,540.00 ± 156.52 | 382.09 ± 45.16$^{*}$ |
| 2% extract | 123.28 ± 14.36 | 1,246.78 ± 143.42$^{**}$ | 1,767.24 ± 194.46$^{**}$ | 375.27 ± 41.45 |
| Chicken manure | | | | |
| 1% extract | 97.14 ± 8.07$^{***}$ | 942.17 ± 109.69$^{**}$ | 1,331.17 ± 125.48$^{**}$ | 298.59 ± 34.03$^{***}$ |
| 2% extract | 95.38 ± 9.45$^{***}$ | 964.44 ± 95.81$^{**}$ | 1,349.49 ± 147.18$^{**}$ | 294.49 ± 30.52$^{***}$ |
| Pig manure | | | | |
| 1% extract | 82.57 ± 9.06$^{***}$ | 804.23 ± 78.93$^{***}$ | 1,139.83 ± 126.60$^{***}$ | 254.92 ± 27.26$^{***}$ |
| 2% extract | 87.52 ± 8.61$^{***}$ | 865.46 ± 99.07$^{***}$ | 1,253.08 ± 132.99$^{***}$ | 270.83 ± 32.37$^{***}$ |

* significant different ($p < 0.10$) from Bristol medium

** significant different ($p < 0.05$) from Bristol medium

*** significant different ($p < 0.01$) from Bristol medium

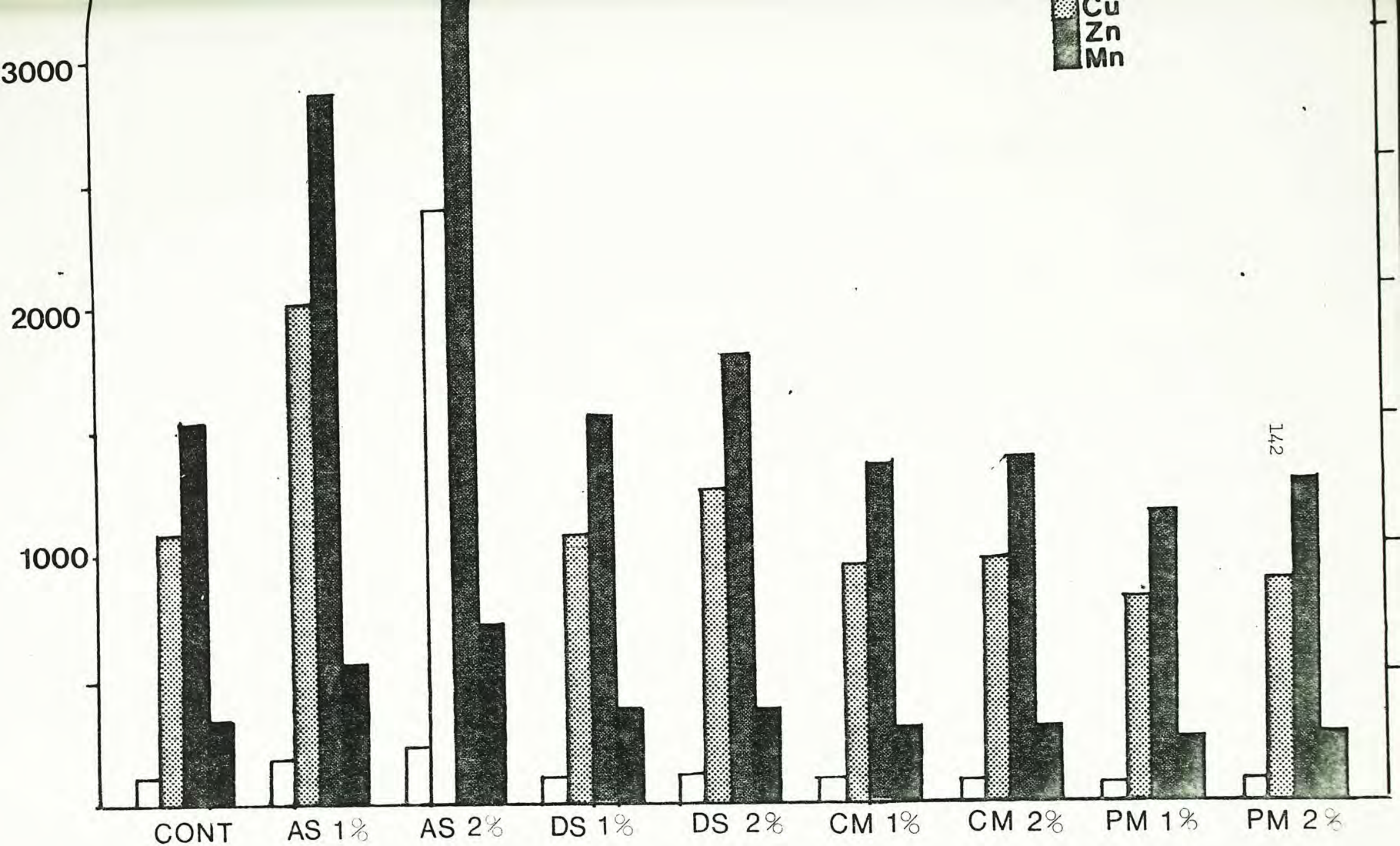




Fig. 6.3 Heavy metal contents (ppm) of harvested exoskeleton of shrimps fed with various waste-grown algae

Table 6.10 Heavy metal contents (ppm) of moulted exoskeletons of shrimps fed with various waste grown algal products.

| | Pb | Cu | Zn | Mn |
|---|---|---|---|---|
| Bristol medium | 193.71±21.51 | 2,017.51±317.98 | 2,724.92±229.02 | 572.75± 58.23 |
| Activated sludge | | | | |
| 1% extract | 514.28±53.57*** | 5,014.82±794.69*** | 7,125.47±759.87*** | 1,518.12±173.92*** |
| 2% extract | 657.97±60.75*** | 6,986.47±929.45*** | 9,564.77±966.07*** | 1,907.36±216.62*** |
| Digested sludge | | | | |
| 1% extract | 346.74±42.39*** | 3,519.21±586.37*** | 4,892.45±493.19*** | 1,448.65±181.77*** |
| 2% extract | 398.57±38.04*** | 4,034.37±635.12*** | 5,621.04±580.91*** | 1,165.09±113.42*** |
| Chicken manure | | | | |
| 1% extract | 280.42±25.37*** | 2,624.31±395.72*** | 3,749.30±383.95*** | 865.44±106.31*** |
| 2% extract | 242.85±21.53*** | 2,497.25±366.53** | 3,629.64±355.96*** | 732.73± 74.18*** |
| Pig manure | | | | |
| 1% extract | 207.90±22.23 | 2,141.25±338.17 | 3,002.87±295.19* | 604.28± 65.95 |
| 2% extract | 225.71±24.45** | 2,450.30±388.24** | 3,329.73±349.92*** | 684.11± 70.90*** |

* significant different ($p > 0.10$) from Bristol medium

** significant different ($p > 0.05$) from Bristol medium

*** significant different ($p > 0.01$) from Bristol medium

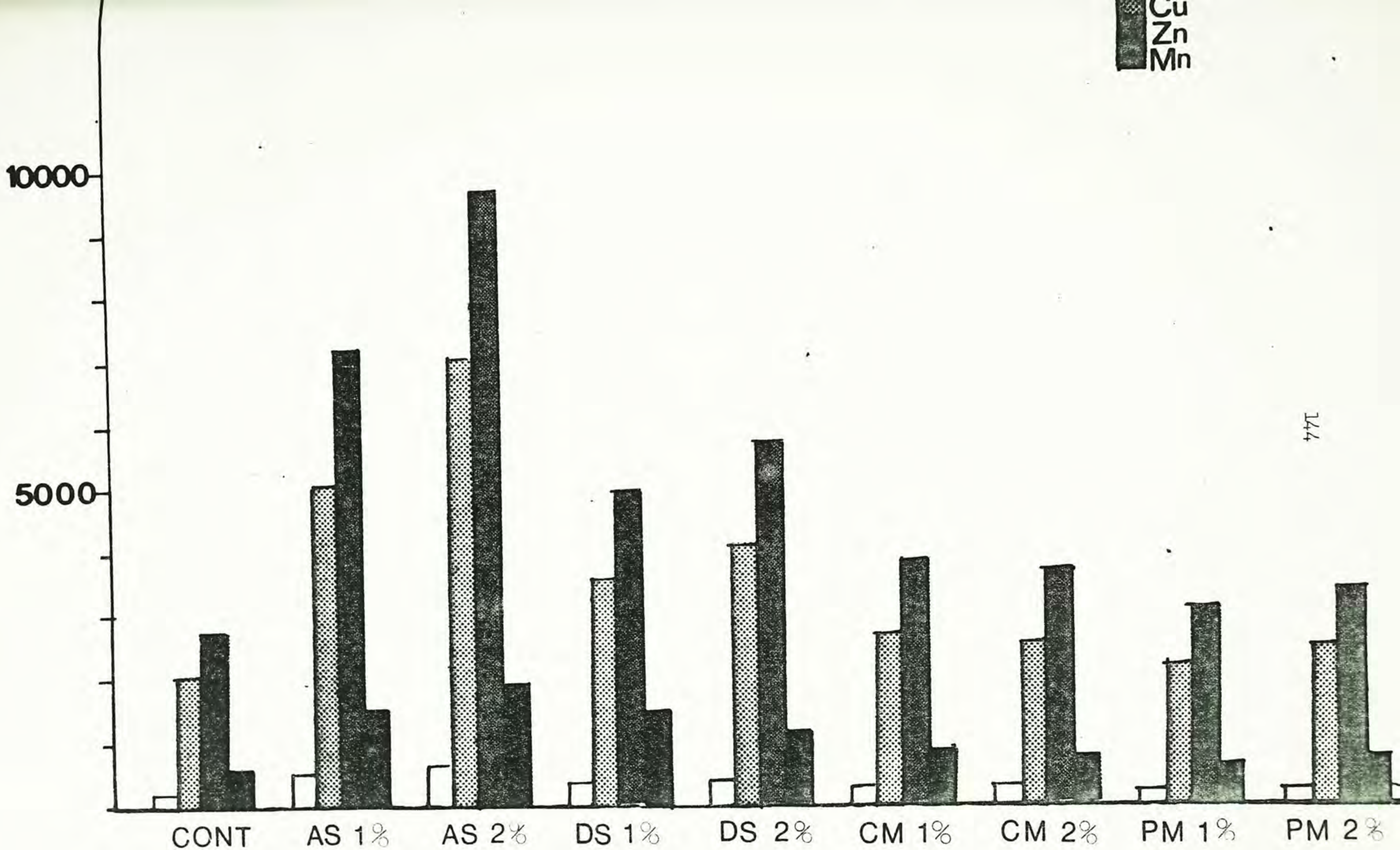


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Fig. 6.4 Heavy metal contents (ppm) of moulted exoskeleton of shrimps fed with various waste-grown algae exoskeleton > harvested meat. Concerning with different kinds of heavy metals, it was found that Zn > Cu > Mn > Pb. Among the four wastes, the highest heavy metal content was still found in shrimps fed by activated sludge grown algae, followed by those fed by digested sludge, chicken manure and pig manure.

In order to correlate the heavy metal contents between the algal products and different portions of shrimps, linear regression analysis was conducted and corresponding coefficients of correlation were obtained (Table 6.11). Results showed that Pb, Cu and Zn contents of the shrimps were significantly correlated with the algal products used to feed the shrimps. Coefficients of correlation between Pb, Cu and Zn contents were found to be 0.85 - 0.94 ($p < 0.01$), 0.94 - 0.99 ($p < 0.01$) and 0.83 - 0.99 ($p < 0.01$) respectively. Mn content was not found to be correlated with coefficients of correlation ranged between 0.32 - 0.55 ($p > 0.05$).

## 6.4 DISCUSSION

In general, sewage sludges seemed to be more inferior when compared with the animal manures for the purpose of producing edible protein due to their higher heavy metal uptake and lower cell number, oven-dried weight, chlorophyll content and nutritive value. The above results also indicated that digested sludge was better than activated sludge while pig manure better than chicken manure (Section 6.3 of this thesis).

Table 6.11 Results of linear regression analysis of various heavy metal contents between algal products and different portions of harvested shrimps.

Pb

$M = 50.82 + 0.18\ (AP)$ $r = 0.90$ $p < 0.01$

$HE = 55.00 + 0.81\ (AP)$ $r = 0.94$ $p < 0.01$

$ME = 146.40 + 2.25\ (AP)$ $r = 0.85$ $p < 0.01$

Cu

$M = 327.37 + 1.16\ (AP)$ $r = 0.94$ $p < 0.01$

$HE = 585.32 + 0.77\ (AP)$ $r = 0.99$ $p < 0.01$

$ME = 1,528.22 + 2.22\ (AP)$ $r = 0.99$ $p < 0.01$

Zn

$M = 872.70 + 0.72\ (AP)$ $r = 0.91$ $p < 0.01$

$HE = 885.37 + 0.77\ (AP)$ $r = 0.99$ $p < 0.01$

$ME = 2,383.57 + 2.11\ (AP)$ $r = 0.91$ $p < 0.01$

Mn

$M = 175.27 + 0.88\ (AP)$ $r = 0.55$ $p > 0.05$

$HE = 192.70 + 0.93\ (AP)$ $r = 0.55$ $p > 0.05$

$ME = 699.60 + 1.68\ (AP)$ $r = 0.30$ $p > 0.05$

M : meat
HE : harvested exoskeleton
ME : moulted exoskeleton
AP : algal product
r : coefficient of correlation

At sublethal concentrations, incorporation of heavy metals in diets retarded body growth and reproduction of organisms. Development of estuarine mudcrab (Rhithropanopeus harrisii) was delayed by Zn and Pb (Benijts and Benijts, 1975). Hg, Cu, Pb, Cd and Zn affected reproduction rates of marine ciliates (Euplotes nannus) (Leland and Luoma, 1977). Hg delayed molting and induced morphological deformities in grass shrimp (Palaemonetes pugio) (Shealy and Sandifer, 1975).

Pb, Cu, Zn and Mn were chosen for measurements in this study because these heavy metals are associated with each other as natural impurities (Thomann et al., 1974) or as alloys (McKee and Wolf, 1963). Cu and Zn are essential components of metello-enzymes (Fisher, 1975). Pb inhibites biological systems and competitively infere with Cu and Zn (Kagi and Vallee, 1961; Hill et al., 1963; Niklowitz and Teager, 1973; Ribas-ozonas et al., 1970). Experimental results of the present study showed both biomagnification and bioelimination of heavy metals. According to Table 6.11, Pb, Cu and Zn contents of shrimps were found to be dependent of algae used for feeding them. The three metals Pb, Cu and Zn, were found in linear rrgression with higher values of coefficient of correlation (0.85 - 0.99) and a positive slope of regression (+0.18 - +2.11). This showed that the higher heavy metal contents in the food, the higher the level of accumulation in the body of consumer. Thus, heavy metal levels were biomagnified through trophic levels. This coincided with a study conducted by Amiard and Foulquier (1978). $Co_{60}$ of Daphina sp. (consumer) was found strongly correlated (r = 0.72 - 0.86) with that of Chlorella sp. (producer). Food was thus recognized as an important source of contamination for herbivorous and carnivorous organisms (Amiard, 1979a, 1979b; Amiard and Amiard, 1975). Mn and Zn were also found biomagnified in shrimps fed with unicellular green algae cultured in sewage sludge extracts. However, Cu was not found biomagnified while Pb was not measured (Tam, 1979).

On the other hand, moulted exoskeleton during culture period had significantly ($p < 0.05$) higher heavy metal contents than that of harvested exoskeletons. This indicated that the shrimps, by means of periodic molting of exoskeleton, could eliminate the toxic heavy metals they accumulated. This coincided with that found by Bertine and Goldberg (1972).

Decrease in heavy metal accumulation was found to be related to increase in size of the shrimps. In this study, the lowest heavy metal concentrations and the highest increase in body weight were found in shrimps fed with algae grown on pig manure extract. On the other hand, the highest heavy metal contents and the lowest increase in body weight were found in shrimps fed with algae grown on activated sludge extract (Tables 6.6, 6.8 - 6.10). New tissues were being incorporated at a greater rate than heavy metals could be actively transported into the tissues to establish a steady-state concentration, i.e. dilution by growth (Cross et al., 1973; O'Rear, 1971). Decreased heavy metal concentrations with size had also been concluded to be related to a decrease in the percentage composition of viscera which was known to be efficient in accumulation of heavy metals (Chernolf and Dooley, 1979). Thus determination of whole body heavy metal contents were critical to the study of biomagnification because predators consumed entire prey, not selected organs (Davis and Boyd, 1978; Goodyear and Body, 1972). Consequently, whole body metal concentrations from both contaminated and uncontaminated sites were of increasing importance to investigators.

The present experiment only investigated on food chain of two levels (i.e. primary producer and primary consumer). Further studies involving more trophic levels are desirable in order to quantify whether organisms on higher trophic levels are affected.

# CHAPTER SEVEN

## Heavy metal transfer in a simulated, terrestrial, two-level food chain (*Brassica parachinensis*, *Rhaphanus sativus* var. *longipinnatus* and *Pieris canidia*)

### 7.1 INTRODUCTION

Heavy metal concentrations in soils were affected by various human activities including cultivation, drainage, fertilization and waste disposal (Allaway, 1968). Such rise in levels could be a hazard to the natural ecosystem if accumulation resulted through food chain.

Various physico-chemical and biological factors are known to influence the translocation of metals from soil to plants and from plants to animals. Mere presence of a metal in soil does not necessarily indicate its availability to plants grown in it. Soil type, solubility of metal, form and its complex formation with chemical and biological constituents of soils affect the accumulation of the metals by plants (Boawn, 1971; Haghiri, 1973; John, 1973; Lisk, 1971; Page et al., 1972). Besides, unknown mechanisms in the plants also play a role in reducing the level of accumulation of metals (Lisk, 1971).

In some studies, even though increase in heavy metal contents in several fold was found in plant tissues grown from sewage sludge-amended soil, no symptoms of phytotoxicity could be found (Hinesly et al., 1972, 1974; Jones et al., 1973).

When heavy metal contaminated plants were consumed by animals, theoretically heavy metals would be transferred up the food chain. Braude et al. (1978) had conducted a series of experiments in food chain study. They warned that the food chain could be contaminated unless stringent measures were taken to prevent it.

Contradictory opinions were held by other researchers. The most highly contaminated species were the primary elements in the food chain, particularly macrophytes. Contamination levels in animal species decreased directly with increasing zoological complexity which in turn generally corresponded to higher trophic level (Amiard and Foulquier, 1978). This means that contaminants incorporated into the animals through dietary intake were eliminated out of the body by certain mechanisms . Such homeostatic mechanisms limited the introduction of harmful amounts of heavy metals into the the animal body (Evans et al., 1979). However, these mechanisms were poorly understood (Underwood, 1971).

Accumulation of heavy metals in terrestrial food chain studies had been revealed by various researchers. In a study comprising digested sludge → corn → pheasants, Cd and Cu were found accumulated in kidney and liver of the bird (Hinesly et al., 1976). Rats accumulated three times radioactive Mn in kidney, liver and duodenum more than that in forage used to feed them (Lassiter et al., 1972). Calves incorporated radioactive Zn from contaminated corn plants (Neathery, 1972).

Earthworms had been shown to accumulate Pb and Zn from contaminated soils (Gish and Christensen, 1973; Ireland, 1975, 1976). Pb contaminated earthworms when used to feed Xenopus laevis, the heavy metal was found retained in the toad (Ireland, 1977). Besides, it was found that increase in Cd in animal tissues depended upon the increase of this metal in the vegetation (Sharme and Shupe, 1975).

Evidences supporting contradictory opinions were also present. In a food chain study comprising sewage sludge $\rightarrow$ corn, sorghum and soyabeans $\rightarrow$ rats, differences observed in tissue concentrations of Cu, Fe and Mn were so slight as to be within the range of elements normally encountered (Miller and Boswell, 1976). Heavy metal levels of liver and kidney of cattle grazed on sewage irrigated pasture were not significantly raised (Evans et al., 1979).

In this experiment a terrestrial food chain was simulated in order to investigate the heavy metal transfer. The simulated food chain comprised the wastes as nutrient level, Flowering Chinese Cabbage, Brassica parachinensis, and Chinese Radish, Rhaphanus sativus var. longipinnatus, as primary producer level and Common White Butterfly, Pieris canidia, as primary consumer. Leaves of the vegetables harvested from waste-amended soils were used to feed caterpillars of the butterfly. Heavy metal contents of both the producer and consumer were compared.

## 7.2 MATERIALS AND METHODS

### A Collection and treatment of samples of sewage sludges and animal manures

The sludge samples were obtained from two sites : activated sludge from the Sewage Treatment Plant at The Chinese University and digested sludge from Shek Wu Hui Sewage Treatment Palnt. The manure samples including chicken manure and pig manure were collected from farms in the New Territories. All the samples were dried under the sun for two weeks and grinded by a hand-driven grinder. They were then sieved through a 2 mm sieve before use.

### B Cultivation of vegetables

Regular tanks, measuring 60 cm x 40 cm x 20 cm, filled with freshwater sand, were used for the growth of the vegetables. Application of samples of sludges and manures to the tanks was based on a weight per unit area ratio. Rates of application chosen were 2.5 and 5.0 kg/m$^2$ (Table 7.1). Tanks after applied with the wastes were irrigated daily with water for one week before seeds of Flowering Chinese Cabbage (*Brassica parachinensis*) and Chinese Radish (*Rhaphanus sativus*, var. *longipinnatus*) were sown.

About fifty seeds were sown to each tank and three weeks later, only four of the strongest seedlings were chosen for further growth, the vegetables were harvested five weeks later.

Table 7.1 Abbreviations of treatments in this study

| | |
|---|---|
| CONT | Control group, without any wastes added |
| | Activated sludge added, at a rate |
| AS2.5 | 2.5 $kg/m^2$ |
| AS5.0 | 5.0 $kg/m^2$ |
| | Digested sludge added, at a rate |
| DS2.5 | 2.5 $kg/m^2$ |
| DS5.0 | 5.0 $kg/m^2$ |
| | Chicken manure added, at a rate |
| CM2.5 | 2.5 $kg/m^2$ |
| CM5.0 | 5.0 $kg/m^2$ |
| | Pig manure added, at a rate |
| PM2.5 | 2.5 $kg/m^2$ |
| PM5.0 | 5.0 $kg/m^2$ |

Since there were four samples of wastes and each had two rates of application, therefore, there were totally nine different treatments, including the control, for each kind of vegetable.

C Feeding butterfly caterpillars with the vegetables

The butterfly chosen for this study was Common White Butterfly (Pieris canidia) belonging to Family Pieridae of Order Lepidoptera (Hill et al., 1978). It is a common, widespread and abundant crop pest in Hong Kong feeding on the leaves of most local cultivated Cruciferae, almost all of which are vegetable crop plants. Breeding of this animal is throughout the year with a considerable number of generations (Hill and Cheung, 1978).

The two vegetables grown in this experiment belonged to Family Cruciferae, therefore served as larval host plants for the butterfly. Eggs of the butterfly were collected in the campus of The Chinese University at March (breeding season). Collected eggs were kept in petri dishes lined with moistened filter papers. Larvae hatched after few days and started to feed on their eggshells and the host plant. When the larvae reached 3 mm in length, they were ready for the feeding experiment.

Young leaves of various waste-grown vegetables were cut and placed on petri dishes lined with moisten filter papers. Caterpillars were transferred onto the petri dishes which were then placed in a cool shaded place in the laboratory.

Precautions were taken not to allow ants to reach the petri dishes. Leaves were replenished daily for a period of two weeks prior measurements and analysis of the caterpillars.

D Analysis of soil

Before the sowing of seeds, soil samples were taken at 2 cm below surface for analysis. They were air-dried and passed through a 2 mm sieve before the following analysis were carried out : pH (pH meter, 10 gm goil : 50 ml distilled water), conductivity (conductivity meter, 10 gm soil : 50 ml distilled water), organic carbon (Walkey and Black, 1934), nitrogen (Kjeldahl digestion followed by ammonia electrode measurement) and extractable phosphate (extractant : 2.5% acetic acid followed by molybdenum blue method, Watanabe and Olsen, 1962). Total heavy metals (mixed acid digestion, $HClO_4 : HNO_3 : H_2SO_4 = 2 : 10 : 1$) and exchangeable heavy metals (extractant : 1 M ammonium acetate solution at pH = 7) were also measured using an atomic absorption spectrophotometer following the methods described by Allen et al. (1974).

E Analysis of vegetables

Heavy metal contents of leaves of the vegetables were determined by mixed acid digestion ($HClO_4 : HNO_3 : H_2SO_4 = 2 : 10 : 1$) and measured by an atomic absorption spectrophotometer.

F Analysis of caterpillars

Fresh and oven-dried weight of caterpillars after two weeks feeding period were measured. Heavy metal contents of the whole animal were determined by mixed acid digestion ($HClO_4 : HNO_3 : H_2SO_4 = 2 : 10 : 1$) and measured by an atomic absorption spectrophotometer.

## 7.3 RESULTS

### A Effect of addition of wastes on edaphic properties (Table 7.2)

a Addition of sewage sludges lowered the pH of the soil while addition of animal manures showed an opposite effect. Activated sludge-added soil was more acidic (5.3 in AS 2.5 soil) than digested sludge-added soil (5.8 in DS 2.5 soil). On the other hand, chicken manure-added soils were more alkaline (7.4 in CM 2.5 soil) than pig manure-added soil (6.5 in PM 2.5 soil).

b Among the four wastes, activated sludge-added soils showed the highest conductivity values (593 µS in AS 5.0 soil). Chicken manure-added soils (334 µS in CM 5.0 soil) and pig manure-added soils (215 µS in PM 5.0 soil) showed immediate values. The lowest value was found in digested sludge-added soils (190 µS in DS 5.0 soil). The control treatment which received none of the wastes showed significantly ($p < 0.05$) lower conductivity level than any of the other treatments. It was because the soil used was freshwater sand which was depleted of any salts.

c The organic carbon contents of all the treated soils were significantly ($p < 0.05$) higher than that of control group. Manured soils showed higher organic

Table 7.2 Edaphic properties of soils treated with various concentrations of wastes.

| | pH (1:5) | Conductivity (1:5) (µS) | Organic carbon (%) | Extractable phosphate (ppm) | Nitrogen (ppm) |
|---|---|---|---|---|---|
| CONT | 6.5 | 15.6 | 0.03 | 67.5 | n.d. |
| AS2.5 | 5.3 | 239.5 | 0.16 | 145.0 | 240 |
| AS5.0 | 5.6 | 593.0 | 0.81 | 232.5 | 500 |
| DS2.5 | 5.8 | 115.5 | 1.06 | 262.5 | 165 |
| DS5.0 | 5.9 | 190.0 | 1.41 | 647.5 | 285 |
| CM2.5 | 7.4 | 97.5 | 1.51 | 970.0 | 217 |
| CM5.0 | 7.9 | 333.5 | 1.65 | 1,565.5 | 525 |
| PM2.5 | 6.5 | 196.8 | 1.34 | 1,172.5 | 410 |
| PM5.0 | 6.9 | 214.8 | 1.88 | 1,337.5 | 635 |

n.d. denoted nondetectable

carbon content than sludge-amended soils. Pig manure (1.88% in PM 5.0 soil) and digested sludge (1.41% in DS 5.0 soil) amended soils had higher organic carbon contents than that of chicken manure (1.65% in CM 5.0 soil) and activated sludge (0.8% in AS 5.0 soil) amended soils respectively.

d Similar results were also noted in extractable phosphate content. Manure-amended soils had higher level (1,566 ppm in CM 5.0 soil and 1,338 ppm in PM 5.0 soil) of extractable phosphate than that of sludge-amended soil (233 ppm in AS 5.0 soil and 648 ppm in DS 5.0 soil). Digested sludge-amended and chicken manure-amended soils had higher level than that of activated sludge- and pig manure-amended soil respectively.

e Since freshwater sand was used for the growth of the vegetables, therefore it was reasonable not to find any nitrogen in the soil of the control treatment. Animal manure-amended soils had higher level of soil nitrogen (635 ppm in PM 5.0 soil and 525 ppm in CM 5.0 soil) than that of sewage sludge-amended soils (500 ppm in AS 5.0 soil). Digested sludge-amended and chicken manure-amended soils were relatively lower in nitrogen level than that of activated sludge-amended and pig manure-amended soils respectively.

B Heavy metal contents in soils treated with various concentrations of wastes

Leaves of the total and exchangeable contents of heavy metals (Pb, Cu, Zn and Mn) in soils treated with various concentrations of wastes are presented in Fig. 7.1 and 7.2. Mean values of the total and exchangeable heavy metal contents were analysed by a two-way analysis of variance. No significant difference ($p > 0.05$) was found in total heavy metal contents among the wastes. However, concerning with exchangeable heavy metal contents among the wastes, significant difference ($p < 0.05$) was found. Duncan's multiple range test was further applied to test the difference and the results are summarized in Table 7.3. According to such data, activated sludge-amended soils had the highest exchangeable heavy metal contents while pig manure-amended soils had the lowest contents. Digested sludge-amended soils had lower exchangeable heavy metal contents than that of activated sludge-amended soil. while chicken manure-amended soils had higher contents than that of pig manure-amended soils.

C Heavy metal contents in leaf sections of vegetables treated with various concentrations of wastes

The contents of Pb, Cu, Zn and Mn in leaf sections of vegetables treated with various concentrations of wastes are presented in Figs. 7.3 and 7.4. Results showed that Flowering Chinese Cabbage accumulated a higher level of heavy metals than that of Chinese Radish. This was obvious in AS 5.0

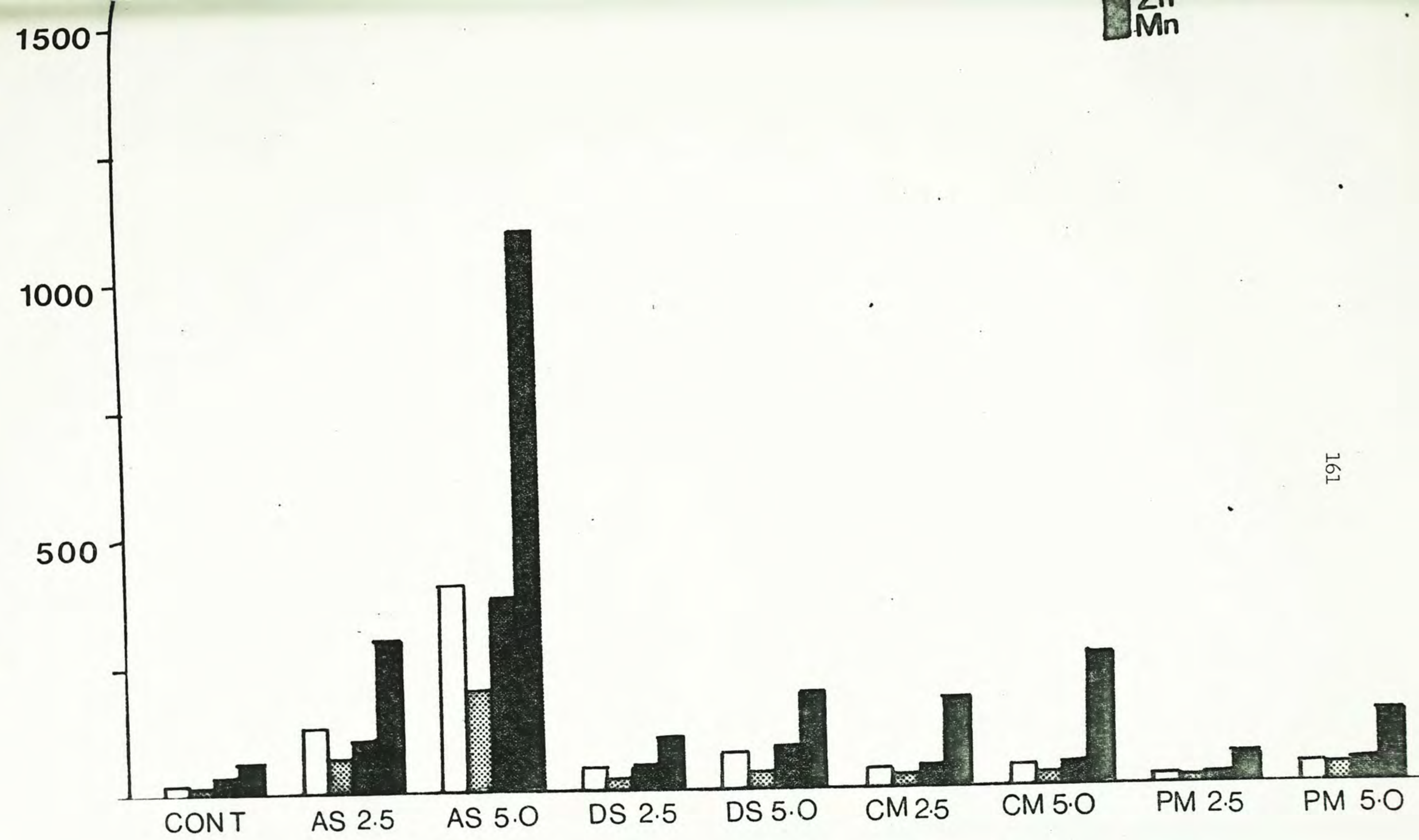


Fig. 7.1 Total heavy metal contents (ppm) in soils treated with various concentrations of wastes

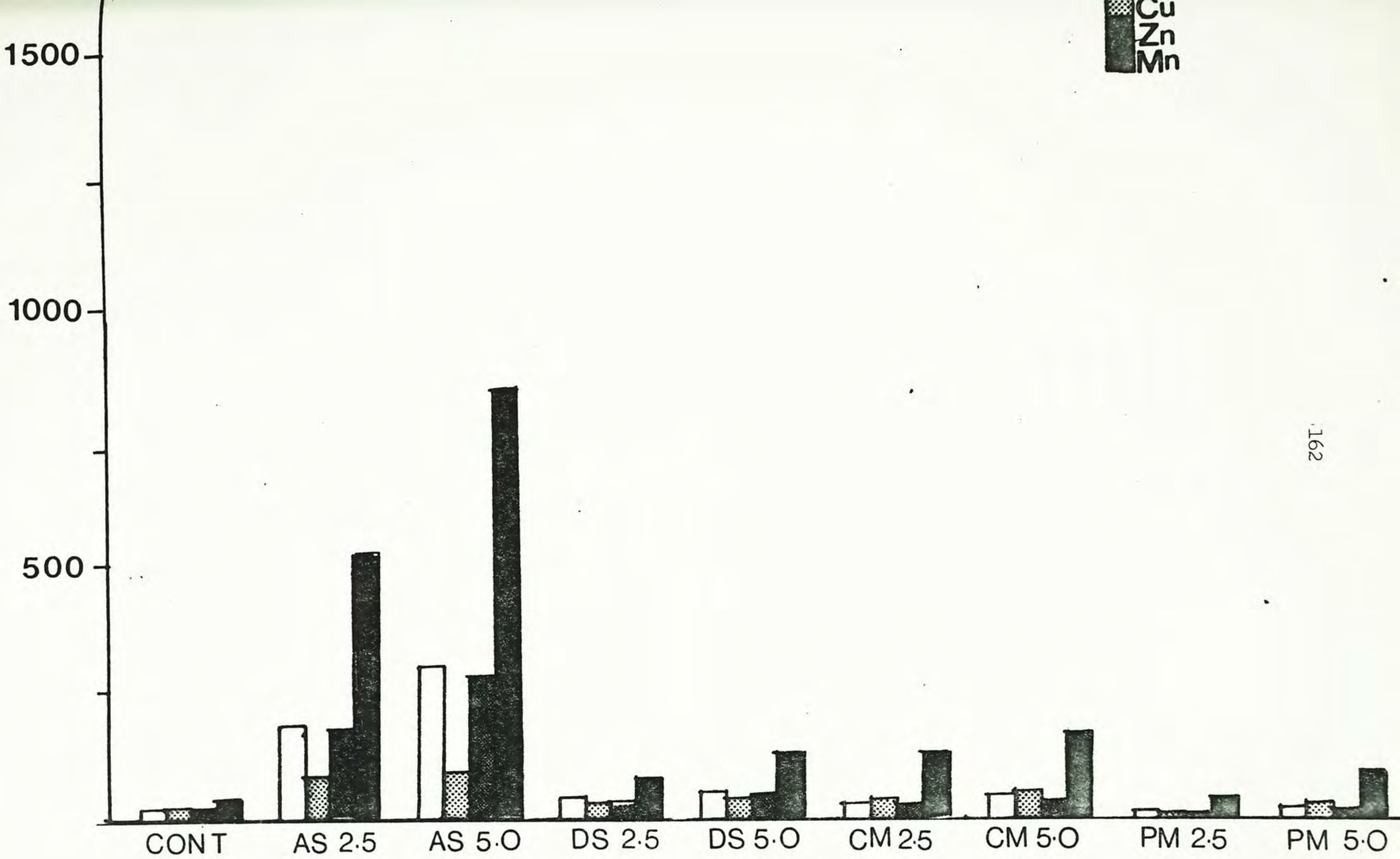


Fig. 7.2 Exchangeable heavy metal contents (ppm) in soils treated with various concentrations of wastes

Table 7.3 Results of Duncan's multiple range test on the levels of total and exchangeable heavy metal contents in the soils treated with various concentrations of wastes.

| Heavy metal | Total | Exchangeable | | | |
|---|---|---|---|---|---|
| Pb | NSD | PM | CM | DS | AS |
| Cu | NSD | PM | DS | CM | AS |
| Zn | NSD | PM | CM | DS | AS |
| Mn | NSD | PM | DS | CM | AS |

Arrangements were in an ascending order from left to right

Items underlined with the same line denoted no significant difference ($p > 0.05$) between them

NSD denoted no significant difference ($p > 0.05$) in two-way analysis of variance

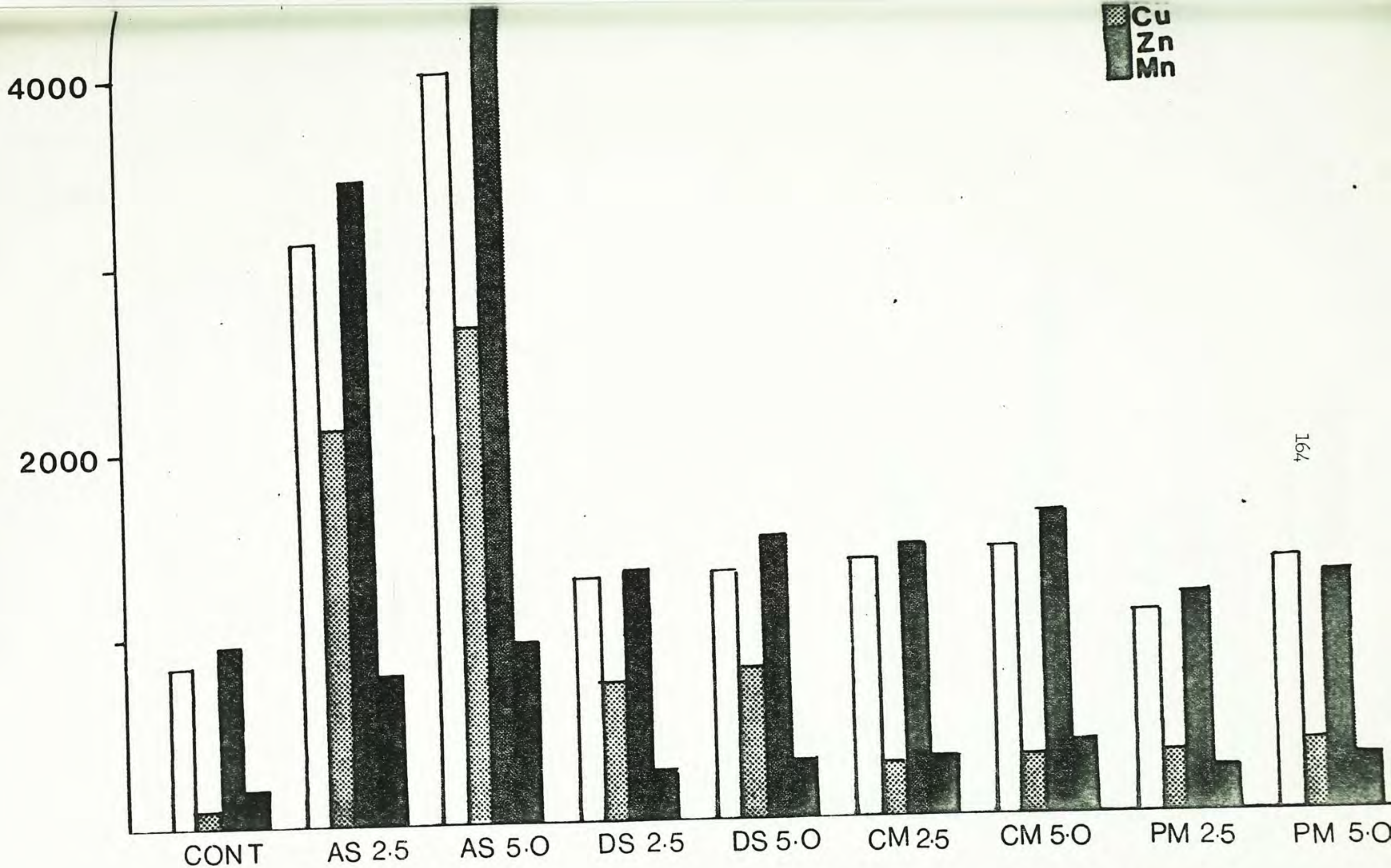


Fig. 7.3 Heavy metal contents (ppm) in leaf sections of Brassica parachinensis treated with various concentrations of wastes

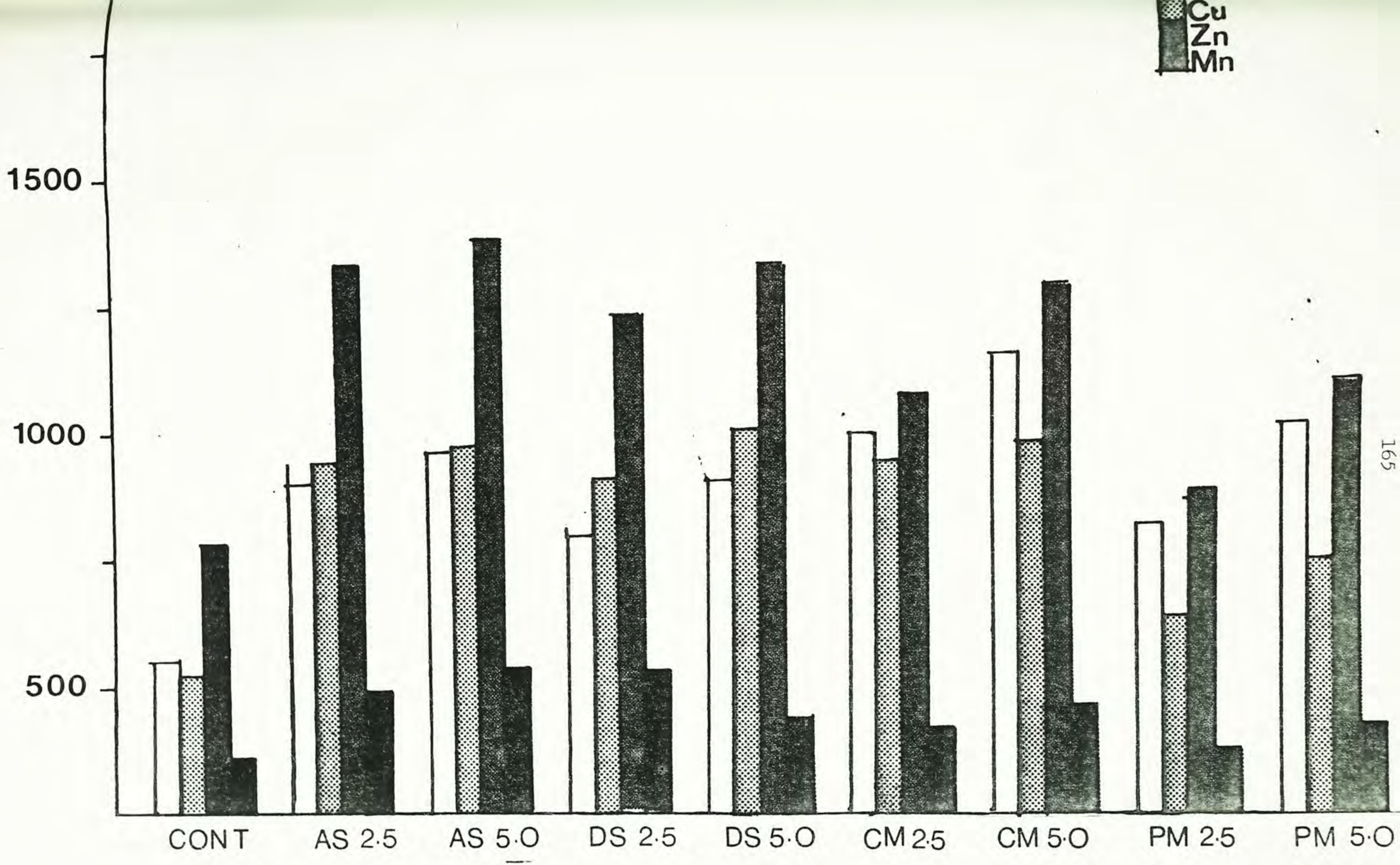


Fig. 7.4 Heavy metal contents (ppm) in leaf sections of Rhaphanus sativus var. longipinnatus treated with various concentrations of wastes

treatment (Pb 5.6 fold, Cu 3.7 fold, Zn 4.0 fold and Mn 6.6 fold). Two-way analysis of variance followed by Duncan's multiple range test were also applied to process the data and the results are listed in Table 7.4. According to the results, the differences in heavy metal contents depended on the type of vegetables and heavy metals. For Brassica parachinensis, activated sludge produced leaves with the highest level of heavy metals while pig mwnure produced the lowest. Digested sludge produced leaves of lower heavy metal levels than that of activated sludge while chicken manure produced leaves of higher heavy metal levels than that of pig manure. As to Rhaphanus sativus var. longipinnatus, the differences in Cu and Zn contents was quite similar with that found in Brassica parachinensis. No significant difference ($p > 0.05$) was found in Mn content. Furthermore, the pattern of difference in Pb content was similar to Brassica parachinensis. Chicken manure produced the highest level of Pb content while digested sludge produced the lowest level. However, when comparing the two kinds of sludges and manures, activated sludge and chicken manure produced leaves of higher Pb than that of digested sludge and pig manure respectively.

D Body weight of caterpillas fed with various vegetables

The fresh weights and oven-dried weights of caterpillars fed with various vegetables are presented in Fig. 7.5. Results shoeed that caterpillars fed with Chinese Radish were

Table 7.4 Results of Duncan's multiple range test on the levels of heavy metal contents in leaf sections of vegetables treated with various concentrations of wastes.

| Heavy metal | Concentration $kg/m^2$ | *Brassica parachinensis* | *Rhaphanus sativus* |
|---|---|---|---|
| Pb | 2.5 | PM DS CM AS | DS PM AS CM |
| | 5.0 | PM DS CM AS | DS AS PM CM |
| Cu | 2.5 | CM PM DS AS | PM DS CM AS |
| | 5.0 | CM PM DS AS | PM CM AS DS |
| Zn | 2.5 | PM DS CM AS | PM CM DS AS |
| | 5.0 | PM DS CM AS | PM CM DS AS |
| Mn | 2.5 | PM DS CM AS | NSD |
| | 5.0 | PM DS CM AS | |

Arrangements were in an ascending order from left to right

Items underlined with the same line denoted no significant difference ($p > 0.05$) between them

NSD denoted no significant difference ($p > 0.05$) in two-way analysis of variance

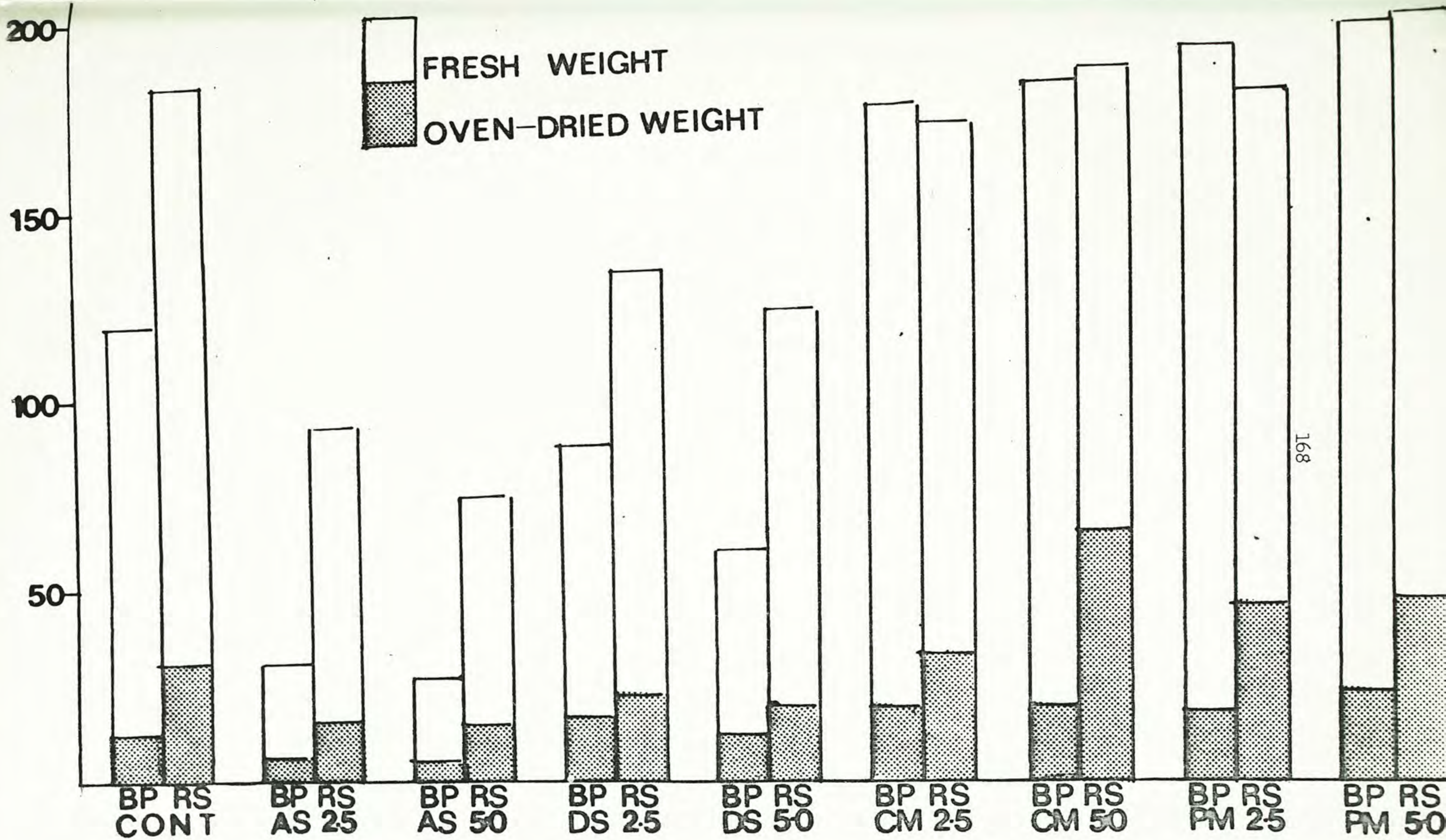


Fig. 7.5 Fresh weights and oven-dried weights (mg) of caterpillars fed with leaves of various vegetables. BP denoted Brassica parachinensis, RS denoted Rhaphanus sativus var. longipinnatus

heavier than those fed with Flowering Chinese Cabbage. Among the four wastes, the average fresh weights of caterpillars fed with Chinese Radish in decending order were pig manure (196 mg in PM 5.0 treatment), chicken manure (186 mg in CM 5.0 treatment), digested sludge (122 mg in DS 5.0 treatment) and activated sludge (75 mg in AS 5.0 treatment).

E Heavy metal contents in caterpillars fed with various vegetables

The contents of Pb, Cu, Zn and Mn in caterpillars fed with various vegetables are presented in Figs. 7.6 and 7.7. Results showed that caterpillars fed with Flowering Chinese Cabbage accumulated a higher level of heavy metals than that fed with Chinese Radish. This was obvious in AS 5.0 treatment (Pb 6.7 fold, Cu 6.0 fold, Zn 6.8 fold and Mn 5.7 fold).

Two-way analysis of variance followed by Duncan's multiple range test were also applied to process the data and the results are listed in Table 7.5. The difference in heavy metal contents of caterpillar fed with Brassica parachinensis showed that caterpillars fed with activated sludge-grown leaves accumulated significantly higher ($p < 0.05$) level of heavy metals than any other waste-grown leaves. Such difference was further magnified in the group fed with Rhaphanus sativus var. longipinnatus. Accumulation of heavy metals in decending order was activated sludge, digested sludge, chicken manure and pig manure (Table 7.4).

In order to correlate the heavy metal contents between

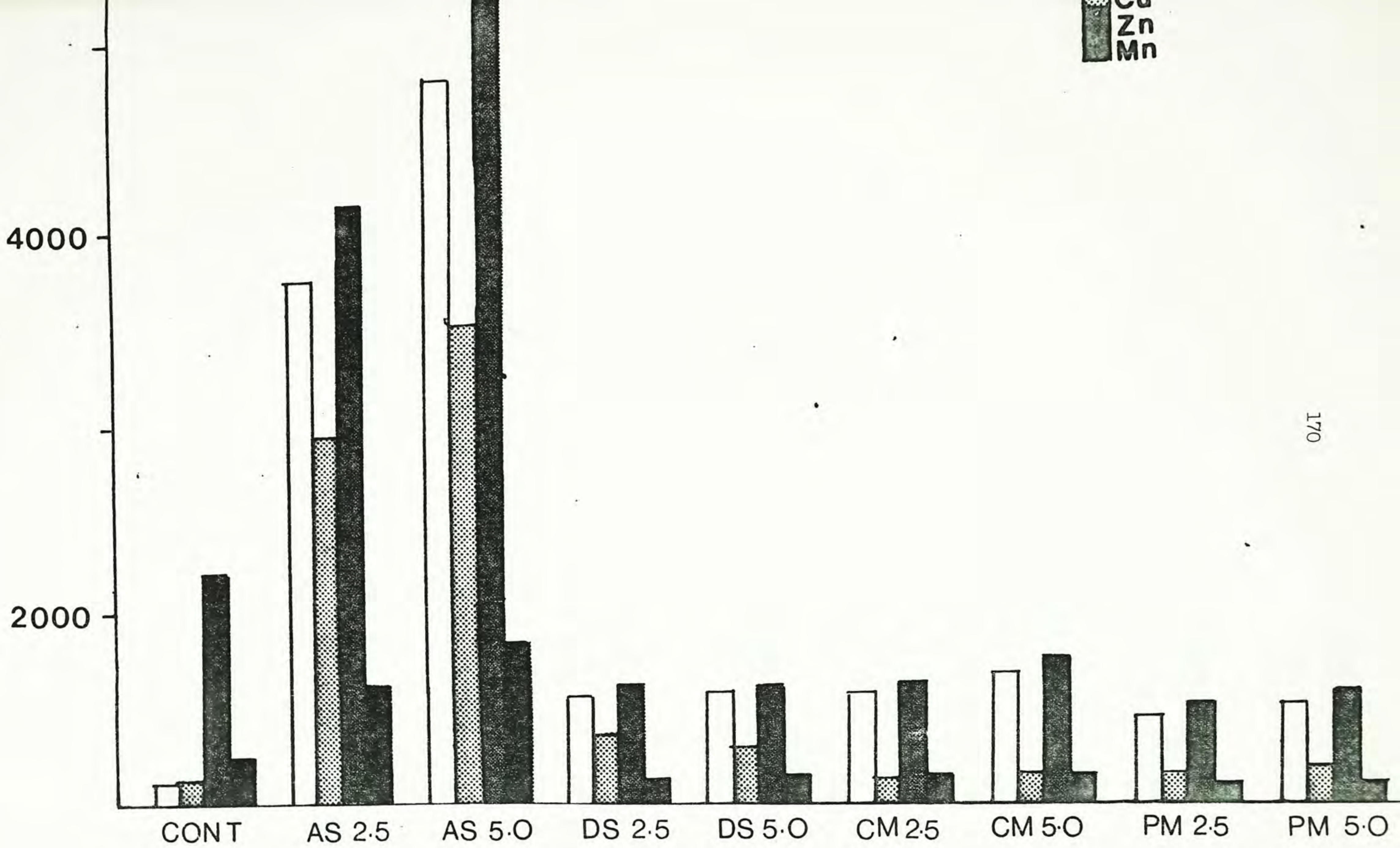




Fig. 7.6 Heavy metal contents (ppm) in caterpillars fed with various leaves of Brassica parachinensis

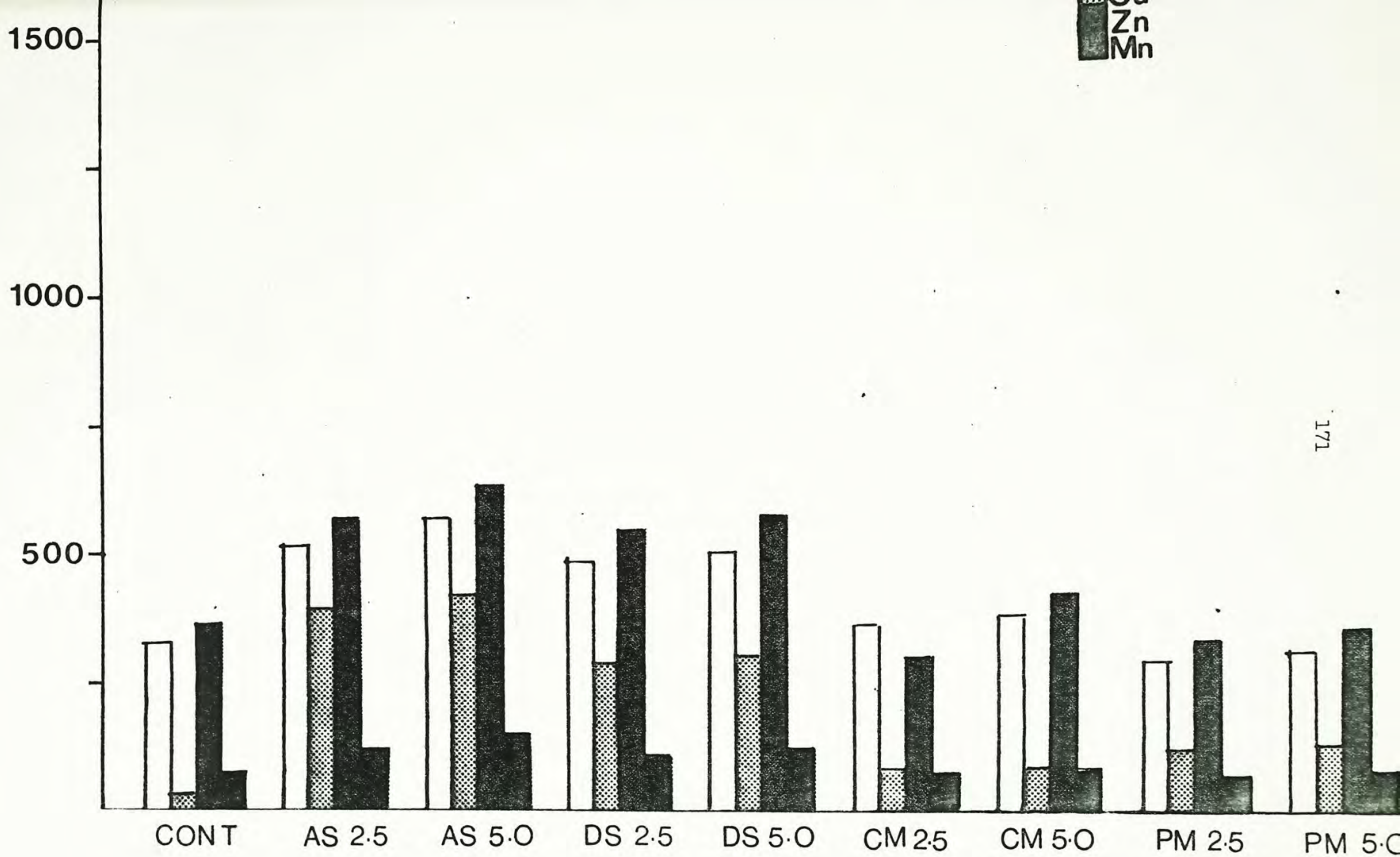


Fig. 7.7 Heavy metal contents (ppm) of caterpillars fes with various leaves of Rhaphanus sativus var. longipinnatus

Table 7.5 Results of Duncan's multiple range test on the levels of heavy metal contents in caterpillars fed with various vegetables.

| Heavy metal | Concentration $kg/m^2$ | Brassica parachinensis | Rhaphanus sativus |
|---|---|---|---|
| Pb | 2.5 | PM DS CM AS | PM CM DS AS |
| | 5.0 | PM DS CM AS | PM CM DS AS |
| Cu | 2.5 | CM PM DS AS | CM PM DS AS |
| | 5.0 | CM PM DS AS | CM PM DS AS |
| Zn | 2.5 | PM DS CM AS | PM CM DS AS |
| | 5.0 | PM DS CM AS | PM CM DS AS |
| Mn | 2.5 | PM DS CM AS | PM CM DS AS |
| | 5.0 | PM CM DS AS | PM CM DS AS |

Arrangements were in an ascending order from left to right

Items underlined with the same line denoted no significant difference ($p > 0.05$) between them

NSD denoted no significant difference ($p > 0.05$) in two-way analysis of variance

Table 7.6 Results of linear regression analysis of various heavy metal contents between leaves and caterpillars.

Pb

| | | |
|---|---|---|
| CAT(BP) = -933.63 + (1.18)BP | $r = 1.00$ | $p < 0.01$ |
| CAT(RS) = 361.99 + (0.08)RS | $r = 0.14$ | $p > 0.05$ |

Cu

| | | |
|---|---|---|
| CAT(BP) = -167.50 + (0.98)BP | $r = 0.99$ | $p < 0.01$ |
| CAT(RS) = -133.10 + (0.56)RS | $r = 0.58$ | $p > 0.05$ |

Zn

| | | |
|---|---|---|
| CAT(BP) = -708.23 + (1.09)BP | $r = 0.96$ | $p < 0.01$ |
| CAT(RS) = 53.52 + (0.45)RS | $r = 0.83$ | $p < 0.05$ |

Mn

| | | |
|---|---|---|
| CAT(BP) = -107.98 + (0.96)BP | $r = 0.96$ | $p < 0.01$ |
| CAT(RS) = 14.63 + (0.44)RS | $r = 0.82$ | $p < 0.05$ |

CAT(BP) = Caterpillar fed on Brassica parachinensis

CAT(RS) = Caterpillar fed on Rhaphanus sativus var. longipinatus

BP = leaves of Brassica parachinensis

RS = leaves of Rhaphanus sativus var. longipinatus

r = coefficient of correlation

p = significant level

Levels of heavy metals in caterpillars and vegetables showed that biomagnification effect was masked by bioelimination. Caterpillars even though ingested an enormous amount of the leaves accumulated a lower heavy metal level than that found in the vegetables (Figs. 7.3, 7.4 7.6 and 7.7). This coincided with the others who held contradictory opinions against those supported biomagnification. Primary level in the food chain was the most contaminated and the degree of contamination decreased in higher levels (Amiard and Foulquier, 1978). Elimination of incorporated heavy metal was found in primary consumers which used contaminated crops as food (Evans et al., 1979; Miller and Boswell, 1976).

Elimination of incorporated heavy metals in animals may be carried out by various ways.

Certain organs of the animal might act as barriers to the heavy metals. For quail, duodenum acted as an effective barrier at intermediate levels of exposure, thus prevented internal damage from Cd (Jacob et al., 1974).

Elimination by excretion is a common means. Pb was mainly excreted in the faeces of earthworm (Ireland, 1976). Dietary As, Cd and Pb were excreted in the faeces of squirrels and rats (Sharma and Shupe, 1976).

Detoxifying mechanism is another means of elimination. Accumulation of metals in particular tissues will remove toxic effects from other tissues where toxic biological reactions may take place. Selective absorption of metals by various organs had been found : Pb in calcified tissues, Cd in liver and kidney, As in hair and integument (Kagi and Vallee, 1960; Schnoeder and Tipton, 1948; Smith, 1967).

Beside the elimination process, concentration and presence of the heavy metals also played a role in reduction of uptake. When the metal concentration in the food was too high, the efficiency of absorption could be lowered. It was found that even though Cd level in sewage sludge grown corn was high, Cd level in kidney and liver of animals grazed on it was low. The reason is the low efficiency of absorption of the metal (Arnord et al., 1973; Hinesly et al., 1976; Scott and Thompson, 1971).

Antagonization between metals means that the presence of a particular metal sedates the presence of another metal. Zn was found to have antagonizing effect on Cd on certain vascular tissues and growth of rats (Banis et al., 1969; Ferm and Carpenter, 1967; Gunn et al., 1961; Parizek, 1957).

Insects regulate internal environment by ionic regulation (secretion and reabsorption) in the alimentary canal. Besides, storage excretion is also an important process to eliminate the effect of excess waste materials on the insects (Wigglesworth, 1972). Elimination process in the caterpillars should be through the faeces. Caterpillars feeding on leaves egested a considerable components of the leaves out of the body. Heavy metals were found associated with the lignin-cellulose matrix (Bremner, 1970). Since lignin is highly resistant to decomposition in animal digestive tracts and protects associated cellulose from decomposition. Therefore, lignin-cellulose matrix in plants comprises the major portion of the undigested fraction. Consequently, metals complexed with these compounds may not be available for absorption. Hinesly et al. (1956) had also found that heavy metals ingested by animals as constituents of plant materials might not be as available as those added as soluble inorganic compound.

Analysis on heavy metal contents of the caterpillars was conducted on the whole animal. Further study should be conducted on the analysis of heavy metal in the faeces and perhaps different organs of the animals receiving vegetables grown in heavy metal-contaminated waste materials.

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# CHAPTER EIGHT

## General conclusion

Recycling of waste materials in a densely populated area such as Hong Kong not only ease the pollution problem but re-utilize the enriched nutrients in the waste materials.

Activated sludge and digested sludge contain a lower level of essential nutrients (i.e. nitrogen and phosphorus) but a higher level of toxic heavy metals than chicken manure and pig manure.

Both activated sludge and digested sludge produced lower yield and protein content of the unicellular green alga, *Chlorella pyrenoidosa*, when growing in the aqueous extracts. Animal manures were more suitable for producing single cell protein due to their higher yield and lower heavy metal contents of the algal products. However, cultivation of algae in the waste materials should be encouraged for either *single cell protein* production for animal feed or *purification of wastewaters* due to their high uptake of heavy metals.

Applying chicken manure to agricultural land is a common practice of the local farmers. However, one of the experiments demonstrated that pig manure produced higher yield of Flower Chinese Cabbage, *Brassica parachinensis*, than chicken manure. This indicated that pig manure also has a high potential as a fertilizer.

Recycling of pig manure is highly essential as some of the streams in New Territories are grossly polluted by pig manure.

Due to the high heavy metal uptake in the Flowering Chinese Cabbage when activated sludge and digested sludge were added, it is suggested that sewage sludge should be used as soil additives for non-edible crops. Furthermore, caution should be taken if crops like carrots were planted due to the higher uptake of heavy metals in the root portions.

Using chicken manure as a fish pond fertilizer for rearing freshwater fish is also a general practice in Hong Kong. However, none of the wastes was found satisfactory when adding as supplementary feed for the common carp, Cyprinus carpio, according to the experiment carried out in the laboratory. All the fish died when animal manures were added. It might be due to the high level of readily decomposable organic matter in the animal manures.

Laboratory scale experiment in plastic tanks could not be compared with field studies in fish ponds. In natural environment, nutrients in wastes passed through photosynthetic planktons or heterotrophic micro-organisms before finally consumed by the fish.

An aquatic food chain (Chlorella pyrenoidosa and Palaeomonetes sp.) and a terrestrial food chain (Brassica parachinensis, Rhaphanus sativus var. longipinnatus and Pieris canidia) were simulated to study the heavy metal transfer from the wastes through the food chains.

In general, organisms in different trophic levels had higher yields and lower heavy metal contents when animal manures were used. When analysing the heavy metal contents, it was revealed that both biomagnification and bioelimination existed.

Pb, Cu, Zn and Mn were accumulated in Chlorella cells when grown in waste materials. Algae are known to concentrate heavy metals from their ambient environment. Shrimps fed with these algae also had high contents of Pb, Cu and Zn in their flesh as well as exoskeleton at the end of the culture period. However, the moulted exoskeleton during the culture period also had a high contents of Pb, Cu and Zn suggested that molting might be an important role in discharging the exceeeive toxic heavy metals.

Vegetables grown in waste materials also contained high levels of Pb, Cu, Zn and Mn. However, it was noted that caterpillars fed on the leaves of these vegetables had lower levels of Pb, Cu and Zn. It was suspected that egestion was effective in discharging the excessive metals. However, further analysis on the heavy metal contents in the faeces would be essential in the future before a definite conclusion could be drawn.

The present study on the utilization of sewage sludges and animal wastes for biomass production showed that the four wastes in decending order of preference was : pig manure, chicken manure, digested sludge and activated aludge.

Food chain studies showed that the properties of the wastes materials were closely correlated with the primary producers and the primary consumers. Both biomagnification and bioelimination were noted according to the analysing of heavy metal contents in organisms of different trophic levels.

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